Master's Thesis

An Evaluation of the Floating Cage System for Eastern Oyster (*Crassostrea virginica*) *Aquaculture Production in the North-Central Gulf of Mexico*

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Declaration

I hereby confirm that I am the sole author of this thesis and it is a product of my own academic research.

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Abstract

In recent years, there has been a significant effort to improve production techniques and product quality for the growing demand of premium farm-raised oysters. The increased interest in off-bottom oyster farming along the north-central Gulf of Mexico (GOM) has provided industry leaders with a significant opportunity to target these specialty markets, capturing the strong demand, stable income, and longer growing season of the GOM. With recent advancements of cultured Gulf oysters into the regional and national half-shell oyster market, the need for industry-based research is in high demand. Through a three-factor field analysis, this study investigates optimal production efficiency and quality control methods for off-bottom oyster culture that will allow Gulf oyster farmers to become increasingly competitive in the half-shell market.

This study investigates methods for improving the productivity and efficiency of the Flippable Floating Cage System (FFCS) in the north-central GOM, by comparing three parameters 1) ploidy (diploid vs. triploid), 2) stocking density (125, 150 &175) and 3) desiccation regime (weekly and biweekly for a 24-hour duration). Over a three-month period (Sept - Nov, 2015) the effects of these factors and their interactions are assessed through the response variables of shell dimensions, shell and tissue weight, shell morphology (cup shape, fan shape, condition index), biofouling accumulation, mud worm *Polydora websteri* abundance and oyster percent mortality. While all treatments used in this study produced high-quality oysters suitable for the premium half-shell industry, there were a number of overall trends identified amongst the tested factors that can serve as helpful recommendations for GOM farmers using the FFCS.

This thesis is dedicated to my parents, Mrs. Marlene Bernadette Gamble and Mr. Murray Allen Gamble from whom I have received incredible support, love, and inspiration.

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List of Abbreviations

°C	Degree Celsius
°F	Degree Fahrenheit
δCI	Delta Condition Index
δDSW	Delta Dry Shell Weight
δDTW	Delta Dry Tissue Weight
δSH	Delta Shell Height
δSL	Delta Shell Length
δSW	Delta Shell Width
δWWW	Delta Whole Wet Weight
µAFDW	Mean Ash Free Dry Weight
µCS	Mean Cup Shape
µDWW	Mean Dry Worm Weight
µFS	Mean Fan Shape
µPM	Mean Percent Mortality
ANOVA	Analysis of Variance
AUORDF	Auburn University Oyster Research and Demonstration Farm
AUSL	Auburn University Shellfish Laboratory
CI	Confidence Interval (95.0%)
FFCS	Flippable Floating Cage System
G	Grams
GOM	Gulf of Mexico
HSD	(Tukey‘s) Honestly Significant Difference
PPT	Parts Per Thousand

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1. Introduction

1.1 General Overview

Aquaculture is the fastest growing food-production sector in the world. The production of farmed seafood, including finfish, crustaceans, and molluscs, has grown at an average annual rate of 8.8% over the past three decades (1980-2010) (FAO, 2012). Over this time period, world aquaculture production has increased from 5 to 63 million tonnes annually (FAO, 2012), contributing a mere 9% to the global seafood supply in 1980 to an impressive 40.3% in 2010 (Seafish, 2013). Today, it is estimated that aquaculture supplies more than 50% of seafood consumed globally, with an industry valued at USD $137.7 billion (farm gate value) (FAO, 2014; WWF, 2015).

As global population is expected to increase to 9.3 billion by 2050 (FAO, 2012), the increased dependence on aquaculture based food production will require enhanced management regimes with industry-wide standards. The anticipated growth and strategic importance of aquaculture in global food supply will demand a strong emphasis on seafood safety, natural resource conservation and environmentally responsible aquaculture (ICES, 2009; Getchis & Rose, 2011; FAO, 2015).

The shift to intensive aquaculture has stimulated widespread discussion amongst researchers, managers, consumers, and policymakers regarding seafood safety, industry innovations, environmental sustainability and how the sector can improve management practices in the future. While topics range from the use of antibiotics, disease control, feed-conversion ratios, genetically modified organisms, seafood traceability, eco-labelling, and best management practices, (Dewey, David, & Cheney, 2011) what remains clear is the need for economically viable protein sources that can be efficiently and sustainably produced with minimal impact on the environment (ICES, 2009; Dewey et al., 2011; Hargreaves, 2011).

The cultivation of shellfish, specifically filter feeding bivalves (oysters, mussels, clams, and scallops) is considered both an example of sustainable aquaculture and a legitimate use of the marine environment for food production (Shumway et al., 2003, Hargreaves, 2011; The World Bank, 2013). In 2010, the cultivation of bivalve shellfish made up approximately 26% of the total world aquaculture production volume, with oysters contributing the greatest proportion (FAO, 2012).

Oysters, commonly found in coastal estuaries, have served as an abundant, accessible, and widely popular food item for centuries (Kurlansky, 2006). The cultivation of oysters is amongst the oldest forms of bivalve shellfish culture (Lorio & Malone, 1994) and is therefore a relatively well-established practice. Through industry innovation and a better understanding of husbandry practices, oysters are increasingly being cultivated with a focus on production efficiency, product consistency, and various characteristics associated with the high-quality half-shell oyster market (i.e. oyster aesthetics, regional flavor, freshness, etc.) (Cheney, 2010). In recent years, through extensive applied research, an increased interest, and substantial investment, the high-quality cultured oyster industry has developed in coastal Alabama (USA) (Walton, et al. 2012). The purpose of this study is to provide effective, viable and widely applicable farm management techniques for the emerging cultured oysters in the north-central GOM.

1.2 Research Aims and Objectives

This research aims to provide oyster farmers with tangible farm management techniques to enhance growth performance and quality control for oysters grown in flippable floating cage systems (FFCS). This study examines the performance of oysters grown in the OysterGro™ (FFCS), a culture gear that is relatively new to the off-bottom oyster industry in the north-central GOM. There are three factors that a farmer can control that may enhance the quality of their product: oyster ploidy, stocking density, and desiccation 'flip regime', all of which have been previously shown to affect off-bottom oyster production (Allen, Gaffney & Ewart, 1993; Creswell & McNevin, 2008; Mallet, Carver & Hardy 2009, Walton et al. 2012). For this project, the overarching question at hand is how the effects and interactions of these three factors impact oysters grown in the FFCS.

Shellfish researchers have been working alongside the cultured oyster industry to help improve crop survival, increase growth rates and overall product quality. Through genetic

processes and selective breeding techniques, shellfish researchers have developed the induced triploid oyster (Allen, Downing & Chew, 1989) which has become widely used throughout the cultured oyster industry (Cheney, 2010). Triploidy is a condition by which the animal retains three sets of chromosomes (3n, triploidy), rather than the usual two sets (2n, diploidy), leading to a sterile oyster known to have improved growth rates, larger meat yields and consistent quality during spawning seasons (Nell, 2002). While natural diploid oysters have been the mainstay of the on-bottom cultured industry, the advancements in spawning technologies and the various performance benefits associated with triploid oysters have facilitated their widespread use within the cultured oyster industry (Allen et al., 1993). The first factor in this study tested the relative performance of diploid and triploid oysters grown in the FFCS.

In addition to ploidy, stocking density (i.e. the number of oysters grown out in gear) is an important factor for oyster quality and consistency of the final market product. A common industry practice involves farmers applying stocking densities of 1/3 container volume to avoid overcrowding and water flow restrictions that can occur as oysters grow (Galtsoff, 1964; Brake, Evans & Langdon, 2003; Comeau, Arsenault & Davidson, 2011, Davis, 2013). While 1/3 rule of thumb can vary according to site-specific farming locations and gear type, the FFCS deployed in the north-central GOM has a recommended stocking density of 150 oysters (per bag) (Davis et al., 2012b). The second factor in this study tested the relative performance of 125, 150 and 175 stocking density to determine how varying the recommended density by 25 oysters (~+17%) would impact growth and quality of oysters in the FFCS.

Finally, biological fouling or 'biofouling' is the accumulation of marine organisms (i.e. algae, barnacles, etc.) on both oysters and oyster culture gear. Many organisms thrive in the productive coastal ecosystems and share habitat with cultured oyster (Brennessel, 2008). The highly productive waters in the north-central GOM offer exceptional growth rate of not only oysters but an abundance of fouling organisms that can debilitate oyster production while creating substantial work for farmers. By periodically desiccating, 'flipping' oysters, farmers can enable air-drying to help control and mitigate the biofouling levels on both oysters and culture gear (Mallet et al., 2009). The third factor in this study tested two flip regimes, where oysters were flipped either on a weekly or biweekly basis for a desiccation duration of ~24 hours.

Furthermore, an additional biofouling factor analyzed the prevalence of a particular biofouling species (mud worm, *Polydora websteri*) under the two alternate flip regimes. The goal of this assessment was to create a widely applicable and effective mud worm mitigation technique when considering the effect and interactions of the three factors; ploidy, stocking density and flip regime for oysters grown in the FFCS (see Manuscript 2).

The overall purpose of this study was to provide oyster farmers with tangible farm management techniques to enhance growth performance and quality control through the effect and interactions of oyster ploidy, stocking density and flip regime for oysters grown in the FFCS.

Specifically, the following hypotheses were tested:

1. Concerning growth, shape and condition of oysters, it is hypothesized that:
   (a) Oyster ploidy will have a significant effect, such that on average triploid oysters will grow significantly larger relative to diploid oysters.
   (b) Stocking density will have a significant effect; such that 150 stocking density will provide optimal performance.
   (c) Flip regime will not have a significant effect on growth, shape and condition of oysters.
2. Concerning biofouling it is hypothesized that:
   (a) Oyster ploidy will not have a significant effect on biofouling.
   (b) Stocking density will not have a significant effect on biofouling
   (c) Flip regime will have a significant effect on biofouling, where flipped oysters will have substantially less biofouling accumulation when compared to biweekly flipped oysters.
3. Concerning the abundance and dry weight of mud worm, *P. websteri* it is hypothesized that:
   (a) Oyster ploidy will not have a significant effect on *P. websteri* abundance.
   (b) Stocking density will not have a significant effect on *P. websteri* abundance.
   (c) Flip regime will have a significant effect on *P. websteri* abundance, such that weekly flipped oysters will have significantly lower *P. websteri* abundance compared to bi-weekly flipped oysters.

1.3 Methodology & Data Collection

This project took place over 84 days (August 25 to November 17, 2015) at Auburn University Shellfish Laboratory (AUSL) on Dauphin Island, Alabama (USA). The OysterGro™ FFCS is the sole grow out technique tested throughout this project, and all cages were deployed at Auburn University Oyster Research and Demonstration Farm (AUORDF), in Portersville Bay, Alabama. At three sampling dates (September 22, October 20, November 17, 2015), a destructive sampling process was used to record the response variables of shell height, shell length, shell width, whole wet weight, dry shell weight, dry tissue weight, and mud worm, *P. websteri*, abundance. Product quality (both shell and meat quality), was measured using shell dimensions (mm) (height, length, width), shell and tissue weight (grams), shell shape (cup and fan ratio), and condition index (weight ratio). Additionally, in the final sampling month (November), average biofouling and average percent mortality was analyzed across all treatments.

1.4 Delineation of Scope

Although there are various gear types used for off-bottom oyster production, such as, the adjustable long-line systems, floating cage system, rack and bag, floating rafts, floating bags, bottom cages, etc. (Appukuttan & Muthiah, 1996; Lavoie, 2005; Mallet et al., 2009), the FFCS was the sole grow out system assessed in this project. Therefore, the results of this study directly apply to the OysterGro™ and other similar FFCS.

The single study site (AUORDF) used throughout the duration of this project means that the results from this study are applicable to farm management techniques specific to the north-central GOM. While many farm sites can offer similar environmental conditions favorable for off-bottom production, each site offers a unique set of advantages and disadvantages that should be considered for successful farm management (Silva et al., 2011). In addition to site-specific environmental conditions, both the duration of grow out and seasonal variations are potentially confounding factors that are outside the scope of this project.

A three-factor test analyzed in this study included the interactive effects of ploidy (diploid, triploid), stocking densities (125, 150, 175) and flip regimes (weekly, biweekly), for oysters grown in the FFCS (for a total of 2 x 3 x 2, or 12 treatment combinations). The data collected are a comprehensive assessment of the final grow out phase, and may not be applicable to the full oyster life cycle.

1.5 Structure of Thesis

The structure of this thesis incorporates two manuscripts; 1) *Critical Analysis for the Off Bottom Floating Cage System: Investigating Optimal stocking density, ploidy comparison, and desiccation regimes* and 2) *Mitigation techniques for mud worm, Polydora websteri, infestation on farm-raised oysters.* Each manuscript includes five main sections (Introduction, Methods, Results, Discussion, Conclusion) followed by a shared final Chapter (Conclusions & Recommendations). These manuscripts were developed from research conducted at Auburn University Shellfish Laboratory (AUSL) on Dauphin Island, Alabama. The results from both studies will be made available to industry members. A strong focus is placed on industry oriented applied science and viable farm level management techniques that directly benefit the development of the off-bottom oyster industry in coastal Alabama. It was beneficial to format the thesis in this manner in order to facilitate reader accessibility and enable future journal publication. This manuscript format was approved by the Coastal and Marine Management Master's program committee.

2 Literature Review

2.1 The Eastern Oyster: Biology, Distribution, Life Cycle

The Eastern oyster *Crassostrea virginica*, (Gmelin, 1791) has a natural distribution along the Atlantic and Gulf coasts of North America from the Gulf of St. Lawrence to the Gulf of Mexico and into the Caribbean (Buroker, 1983; Newball & Carriker, 1983; Andrews, 1991 p.107; National Research Council, 2004).

Figure 2.1 Natural Range for the Eastern Oyster, Crassostrea virginica (VanderKooy, 2012).

Crassostrea virginica is classified under the Kingdom: Animalia, Phylum: Mollusca, Class: Bivalvia, Order: Ostreoida, and Family: Ostreidae (Gaffney, 1996), and is often referred to as the Eastern, American, or Atlantic Oyster (Kurlansky, 2006). While the size, shape, color and taste of Eastern oysters differ depending on their environment (temperature, salinity, depth, food availability, etc.), the irregularly oval shaped shell, smooth shell margins and a large purple-pigmented adductor muscle scar on the interior of the shell are ubiquitous taxonomic distinctions that can help identify *C. virginica* (Kennedy, 2004).

Under natural conditions, adult oysters begin the reproduction cycle with gametogenesis, the production of eggs or sperm (gametes) triggered by the warming water temperatures in spring (March, April, May) (Figure 2.2). Adult oysters are stimulated to discharge their gametes ('broadcast spawn') with the presence of eggs or sperm in the water column, which normally begins once the average water temperature reaches twenty degrees centigrade (20°C, 68° F) (Medcof, 1939; Butler, 1965; Quayle & Newkirk 1989; Kennedy, 2004). The average temperature at which spawning occurs varies from higher to lower latitudes, with northern oysters, typically spawning at temperatures between 12.5° to 20° C (60° to 68° F) and southern oysters requiring temperatures above 20° C (68°F) (Wallace, 2001). Once spawning occurs, it only takes a few hours for fertilization to occur, where the embryos develop into planktonic, trochophore larvae. Roughly 24 - 48 hours after fertilization, the trochophore larvae become veliger larvae through the formation of a 'D-Hinged' shell (Buroker, 1983; Wallace, 2001). The veliger larvae will drift in the water column feeding on phytoplankton, detritus, and bacteria for roughly 2-3 weeks (Kennedy, 1996; Wallace, 2001).

During this planktonic stage, the larval mortality rates are estimated to be 99%, yet the successful veliger larvae that do survive will develop a 'foot', effectively becoming pediveliger larvae (Figure 2.2). At this stage, pediveligers will migrate to the benthos looking to secure their "foot" to a solid surface (oyster shell or 'cultch', pilings, rocks, various hard substrate), where they metamorphose into 'spat' (1 - 24mm). After a successful settlement, spat will continue to feed on suspended particulate matter, where they grow into seed oysters (25mm - 75mm) and eventually adult or 'market' oysters (>75mm) (VanderKooy, 2012).

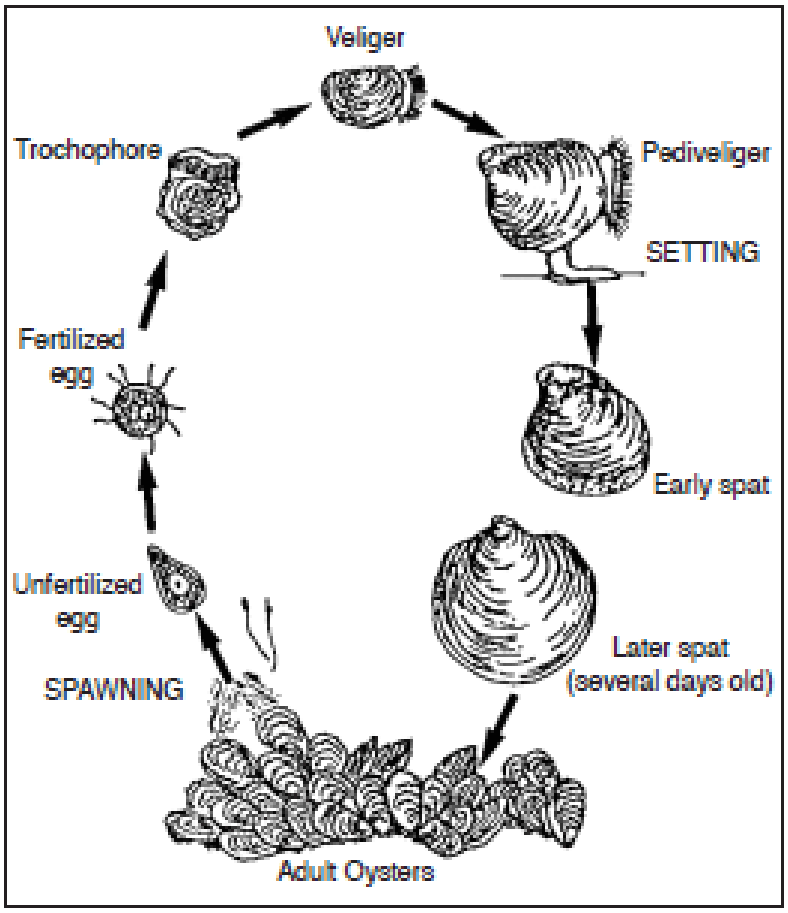

Figure 2.2 The Life cycle of the Eastern Oyster, Crassostrea virginica (Wallace, 2001).

2.2 Oyster Morphology and Environmental Tolerance

The shell of the Eastern oyster consists of two calcareous valves (bivalves), serving as an exoskeleton for the soft internal organs "tissue". The valves are asymmetrical with the left (bottom) valve usually thicker, heavier and cup-shaped (Yonge, 1960; Galstoff, 1964). Eastern oysters cement or 'set' themselves to the substrate on the left valve, leaving the typically flatter, right valve on top. Joined together with a tough elastic hinge ligament at the anterior end of the oyster, the valves are able to remain closed by means of an adductor muscle which forms a water and air tight seal (NOAA, 2007).

With an estimated lifespan of 12-15 years (Kurlansky, 2006) and a vast geographic distribution, Eastern oysters have evolved in various estuarine environments. Past studies have demonstrated that geographically separated populations or 'families' of Eastern oysters, have evolved with molecular and physiological traits that allow them to withstand localized environmental stressors ranging from extreme temperature and salinity variations (Galtsoff,

1964; Burrell, 1986) to the development of disease resistance (Andrews & Hewatt, 1957; Bushek & Allen, 1996a). Several studies have shown that Eastern oysters can tolerate water temperatures from -2° to 36°C (28° to 97° F) and salinity levels from 0 to 42.5 ppt (Butler, 1954; Wallace, 1966; Shumway, 1996; National Research Council, 2004). The Eastern oyster is a highly resilient species capable of adapting to numerous environmental stressors (Reeb & Avise, 1990; Hoover & Gaffney, 2005). However, the story of wild oyster stocks is almost universally one of unsustainable harvest exacerbated by various natural and anthropogenic factors. The extensive demise of the Eastern oyster has occurred across much of its native distribution, resulting in the current status as a functionally extinct species (MacKenzie, 1997b, Lenihan, 1999; National Research Council, 2004).

2.3 Native Oyster Reefs: Evolution of Reef Management

Adult Eastern oysters are a sessile filter feeding organism that can be classified as ecosystem engineers or 'foundation species' (Jones, Lawton & Shachak, 1994). Oysters are found in assemblages referred to as reefs or beds that can extend up to several kilometers in length and tens of meters in height (Kirby, 2004; Lotze et al. 2006). The biogenic reef habitats provide important structural and ecological functions in estuaries throughout the Eastern oyster geographic range (Wells, 1961; Mann, Rainer & Morales-Alamo, 1994; Berqquist, Hale, Baker & Baker, 2006). These extensive calcareous formations were in many ways, the temperate-climate equivalents of coral reefs (Lenihan & Peterson, 1998; Grabowski & Peterson, 2007) as healthy oyster reefs provide habitat for various mollusks, crustaceans, sponges, polychaetes, tunicates, and other resident invertebrates (Wells, 1961; Bahr & Lanier, 1981; Rothschild, Ault, Goulletquer & Heral, 1994). The rich biodiversity provided by healthy reefs provide ideal feeding ground for juvenile fish and mobile crustaceans that together create an enriched ecosystem for the commercial and recreational fisheries embedded in many coastal regions (Coen, Luckenbach & Breitburg, 1999; Breitburg et al., 2000; Harding & Mann, 2001; Peterson, Grabowski & Powers, 2003; Tolley & Volety, 2005).

The extensive natural distribution, historic abundance and close proximity to land allowed the Eastern oyster to become embedded into the cultural makeup of many coastal communities along the Atlantic and Gulf coast of North America (Jacobsen, 2007). However, the regulation and conservation management for oyster reefs have largely consisted of simply

sustaining the fishery by placing limits on the duration of harvest seasons, types of harvest methods and total number of licenses or leases allowed to harvest (Andrews, 1991).

Viewing oyster reefs as merely biological and economic commodities aligns with the traditional and typically destructive one-dimensional method of managing oyster reefs (Grabowski et al., 2012). In recent years, there has been an increasing recognition and appreciation for the irreplaceable ecosystem services (i.e. filtration, denitrification, shoreline stabilization, etc.) provided by oyster reefs, with modern management strategies moving towards ecosystem-wide restoration objectives (Coen et al., 2007; North et al., 2010). A diverse variety of scientific literature demonstrates the numerous non-market ecological systems or 'ecosystem services' offered by healthy oyster reefs. For example, the ability of oysters to remove suspended solids while enhancing water clarity can enable seagrass growth and improve submerged aquatic vegetation (Thayer, Stuart, Kenworthy, Ustach & Hall, 1978; Coen et al., 1999). The same filtration service has been shown to reduce the likelihood of harmful algae blooms (Cerrato, Caron, Lonsdale, Rose & Schaffner, 2004; Newell & Koch, 2004). Healthy oyster reefs can also remove excess nutrients, helping to reduce anthropogenic nitrogen loading in coastal bays (Newell, Cornwell & Owens, 2002). Structurally, oyster reefs can aid in shoreline stabilization serving as natural coastal buffers, capable of absorbing wave energy and reducing erosion (Meyer Townsend & Thayer, 1997; Piazza, Banks & La Peyre, 2005). By combining the biological, ecological and economic benefits associated with healthy oyster reef populations, in becomes hard to dismiss the importance of this species on the estuarine ecosystems (Future of Fish, 2012).

2.4 Wild Fishery and Harvest Methods

As sessile, non-motile organisms, oysters are entirely depended on their surrounding habitat for survival, making them vulnerable to numerous biological (i.e. predation, disease, natural disaster, various abiotic factors, etc.) and anthropogenic sources (i.e. habitat destruction, pollution, over-harvesting, ocean acidification, etc.) (VanderKooy, 2012).

Through a combination of factors, this once-plentiful, ecosystem supporting and regenerative resource is desperately depleted. The demand for oysters, coupled with over-harvesting, environmental degradation, oyster disease, pollution and the overall mismanagement of coastal resource and estuarine ecosystems has led to the dramatic decline of native Eastern oyster populations (Grabowski & Peterson, 2007). Around the world, an

estimated 85 % of oyster reefs have been lost over the past 130 years (Lotze et al., 2006; Beck et al., 2011). The physical oyster reef destruction is often seen as the leading cause for declining oyster harvest and in response to declining reef habitat, oyster shells have been utilized for reef supplementation or 'reef planting' with the intention of accumulating wild 'set' oysters (Lenihan & Peterson, 1998; Vanderkooy, 2012). While restoration projects of both commercial and ecological interest have experienced various levels of success, the rate of sedimentation, predation, and commercial harvest has often outstretched restoration efforts, leaving a stagnant or declining oyster population (Coen & Luckenbach, 2000; Posey, Alphin, Coen, Walters & Wilber, 2006). Within the United States, the once plentiful GOM oyster reefs are estimated at just 20 % of historic abundance and the renowned Chesapeake Bay oyster reefs are estimated at a mere 1 % of former abundance (Future of Fish, 2012).

For human consumption, there are three primary methods (wild, semi-cultured, intensive culture) that humans produce and harvest oysters (Matthiessen, 2001). The 'wild' fishery involves a fisherman searching for uncultivated 'on bottom' native oyster populations with hand tongs, a towed-dredge or simply by hand. The second harvest method is a semi-cultured technique where the harvesters attempt to improve conditions for oyster settlement and survival on private 'beds' or 'leases' (Supan, 2002). Techniques to improve conditions include spreading shell or 'cultch' on the seafloor, controlling natural predators or moving oysters to more favorable oyster grounds as they mature; typically, these oysters are harvested by a mechanical dredge Finally, there is an intensive culture method that involves human involvement from conception to harvest. Intensive oyster culture takes place within the water column and is often referred to as 'off bottom' or 'suspended culture'. Oysters produced in intensive culture methods are typically catered towards the high-value half-shell oyster market, with a strong focus on oyster quality and consistency. In regions where wild oyster populations can no longer support a viable fishery, intensive oyster aquaculture is increasingly utilized as an efficient, viable and sustainable method for producing high-quality oysters.

2.5 Intensive Oyster Aquaculture: Off-Bottom Farming

Modern intensive oyster culture techniques require substantial human intervention including transplanting, stocking, breeding, feeding, washing, grading, protecting etc., however, oyster cultivation, in some form or another, has been practiced for more than 2,000

years (Wallace, 2001; Brennessel, 2008). While recent research suggests the ancient clam gardens (~3000-year-old) along the pacific northwest coast are likely the oldest form of bivalve culture (Groesbeck, Rowell, Lepofsky, Salomon, 2014), the cultivation of oysters can be traced back to the time of the Romans (600AD) (Gunter, 1950; Chew, 1990), with various techniques appearing across a vast cultural, geographic and temporal range. Both, Asia and Europe have long established traditions in oyster culture and many current culture techniques are analogous to early methods in France, Australia, Japan and North America (Brennessel, 2008). Technically. there are only a few main methods used for farming oysters, however, each method has infinite variations as farmers will often adapt unique grow out techniques depending on farm site location, environmental conditions, cultural traditions, and available resources (Wallace, 2001). Throughout the cultured oyster industry, one of the most well-established grow out methods is the off-bottom farming technique.

Off-bottom oyster farming is the culture of oysters in a mesh container (basket, bag, cage, etc.) that is held above the seafloor (Walton et al., 2012). The elevated containers are either floating or suspended in the water column, providing oysters with protection against benthic predators while eliminating burial in sediment, both of which have been shown to decimate wild oyster populations (Gillmor, 1982; Wieland, 2007; Creswell & McNevin, 2008). By elevating oysters to grow in the highly productive surface waters 'photic zone', farmers are introducing oysters to increased water flow and a higher abundance of food availability, enabling significantly higher growth rates and increased survival (Paynter & Dimichele, 1990; Kraeuter, Ford & Cummings, 2007). Additionally, the off-bottom culture technique allows oysters to be grown in areas where the seafloor may no longer be suitable for natural oyster settlement (i.e., mud bottom).

While there are many differences between traditional oyster reef harvesting and oyster aquaculture, one of the main attractions to off-bottom culture techniques is the ability of the farmer to periodically handle their gear to control biofouling and maintain optimal stocking densities (Comeau, 2013; Davis, 2013). The frequency that farmers handle their gear varies according to gear type, seasonal climate conditions, fouling intensity and husbandry practices. By understanding regional environmental factors and adaptive farm management techniques farmers can consistently produce a high-quality product by significantly improving shell shape and appearance while greatly reducing fouling (barnacles, overset oysters, mud worms) (Walton et al., 2012). With the various benefits associated with off-bottom oyster culture

comes a variety of additional factors that require substantial upfront investment and operational cost (i.e. farm sites, permits, cultivation gear, seed oysters, marketing, manual labor, processing fees, etc.) (Comeau et al., 2011; Hargreaves, 2011). By investing in off-bottom oyster aquaculture, farmers are creating a business or 'brand' that identifies and adds value to their oysters in the marketplace, effectively providing a story for consumers to better understand the farming techniques and the environment or 'region' where the oysters were grown (Cheney, 2010; Getchis & Rose, 2011). With a growing demand for premium farm-raised oysters in the specialty shellfish market, strategic regional branding can help establish new and upcoming off-bottom oyster farming businesses in rural coastal communities while creating employment, diversifying the economy and alleviating some of the fishing pressure on wild reefs (Shumway, 2003).

2.6 The Succession of the Gulf of Mexico Oyster Fishery

The United States (US) Gulf States (Texas, Louisiana, Mississippi, Alabama, and Florida) each has unique cultural and economic ties to the historic oyster fisheries in the GOM (Menzel, 1991, VanderKooy, 2012). The US gulf coast oyster industry is a diverse mixture of individual fisheries dependent on the nearshore production of fluctuating natural populations of *C. virginica* (Schlesselman, 1955; Chew, 1982) and is primarily based on 'on-bottom' wild harvest through public reefs, extensive-bottom culture, or privatized bottom-leases. The *C.virginica* oysters from this region contribute a major portion of the annual US eastern oyster production (80-90% in 2012), and are considered amongst the most productive reefs remaining throughout the native habitat range (Vanderkooy, 2012; Matthiessen, 1970b). However, the GOM industry predominately relies on the commodity market, as the traditional oyster product coming from the Gulf States are shucked oysters that have been harvested, processed, and packaged for sale as a cooked, smoked, or raw bulk product (Menzel, 1991; Wirth & Minton, 2004; Cheney, 2010). In comparison to the half-shell oyster industry, the shucked oyster product reaches an average wholesale market price of $3.17 US per pound (National Marine Fisheries Services *as cited in* Walton et al., 2012), while intensive off-bottom oysters destined for the half-shell market can reach wholesale market values of $33.67 US per pound (NOAA, 2009 *as cited in* Walton et al., 2012).

The off-bottom oyster culture technique is a common method used in France, Spain, Japan and Korea and is well established on the Atlantic and Pacific coast of North America,

however, this technique is relatively new to the GOM (Cheney, 2010; Walton et al., 2013). In recent years, through applied research, growing interest, and increased investments, the off-bottom oyster farming industry in coastal Alabama has seen tremendous development. The industry has made significant strides with the first harvest in 2010 estimated at 20,000 oysters ($10,000 wholesale dockside value), 2011-2013 saw slight increases, with best estimates of 300,000 oysters ($150,000 wholesale dockside value) and 2014-2015 harvest reaching 1,000,000 oysters ($500,000 wholesale dockside value) (Walton, W. *pers. comm.,* 2016). The economic incentives of the premium half-shell market, combined with the increased protection and control over harvestable 'crop', makes the off-bottom oyster industry an attractive production method that could help supplement unstable wild oyster harvest while increasing employment and diversifying the local economy. In past decades, the implementation of off-bottom oyster farming in coastal communities along the Atlantic and Pacific coast of North America have allowed for substantial economic development to both the direct and indirect sectors involved (i.e. hatchery, nurseries, farms, processing, distributing, wholesale, retail, etc.). Although off-bottom oyster farming is relatively new to the north-central GOM, the highly productive waters and longer growing season could offer a substantial competitive and economic advantage (10 - 12 month growth cycle) when compared to the east and west coast regions (3 - 5 year growth cycle), suggesting that strategic and collaborative industry wide initiatives combined with ongoing research and increased investments could allow for the advancement of the premium gulf oyster industry.

Past studies in the north-central GOM have focused on industry innovation and improved grow out techniques on a variety of common gear types deployed for off-bottom oyster production (Maxwell & Supan, 2010; Coddington-Ring, 2012; Walton et al., 2012). The applied industry focused research and collaborative initiatives between industry and academia have been a critical component of the recent success in the Alabama off-bottom oyster industry. This project examined the FFCS across three farm-level factors with the goal of providing additional insight for off-bottom oyster farmers in the north-central GOM.

3 Manuscript One

Critical Analysis for the Off Bottom Floating Cage System: Investigating Optimal Stocking density, ploidy comparison, and desiccation regimes

3.1 Introduction

Over the five decades, there have been significant advances in the shellfish aquaculture industry that have enabled farmers to produce safe, consistent, high-quality shellfish (Brennessel, 2008). Throughout the off-bottom oyster aquaculture industry, an emphasis on product quality and consistency has created a high-end niche market for farm-raised oysters, where product distinction, regional flavor profiles, shell aesthetics, freshness, traceability and ease of preparation for the culinary industries has started to play an increasingly important role in farm management and husbandry practices (Cheney, 2010).

Through advances in oyster genetic, innovation in culture techniques and a better understanding of husbandry practices, a farmer entering the intensive off-bottom oyster industry today is inundated with various farm management decisions well before the farming process begins.

By investigating the effectiveness of the Flippable Floating Cage System (FFCS) in Portersville Bay, Mobile County, Alabama, this research aims to provide farm level management techniques for the emerging off-bottom oyster industry in the north-central GOM. The central efforts at Auburn University Shellfish Lab (AUSL) are directed towards applied research for industry solutions, with the hopes of elevating farm-raised Alabama oysters into the competitive, innovative, and lucrative half-shell oyster markets. The intensive, off-bottom oyster initiative is one plausible solution that is currently being implemented through small-scale farming sites, in an attempt to help re-stabilize and bringing new revenue streams to the north-central GOM oyster industry (Walton et al., 2012).

3.2 Methods

3.2.1 Site Description

The Auburn University Shellfish Laboratory (AUSL) on Dauphin Island, Alabama, manages a 65-acre open water shellfish farm common referred to as The Auburn University Oyster Research and Demonstration Farm (AUORDF). The AUORDF is located on a submerged lands riparian rights lease located in Portersville Bay, Alabama (30°21'11.56" N 88°11'28.45W) (see Figure 3.1).

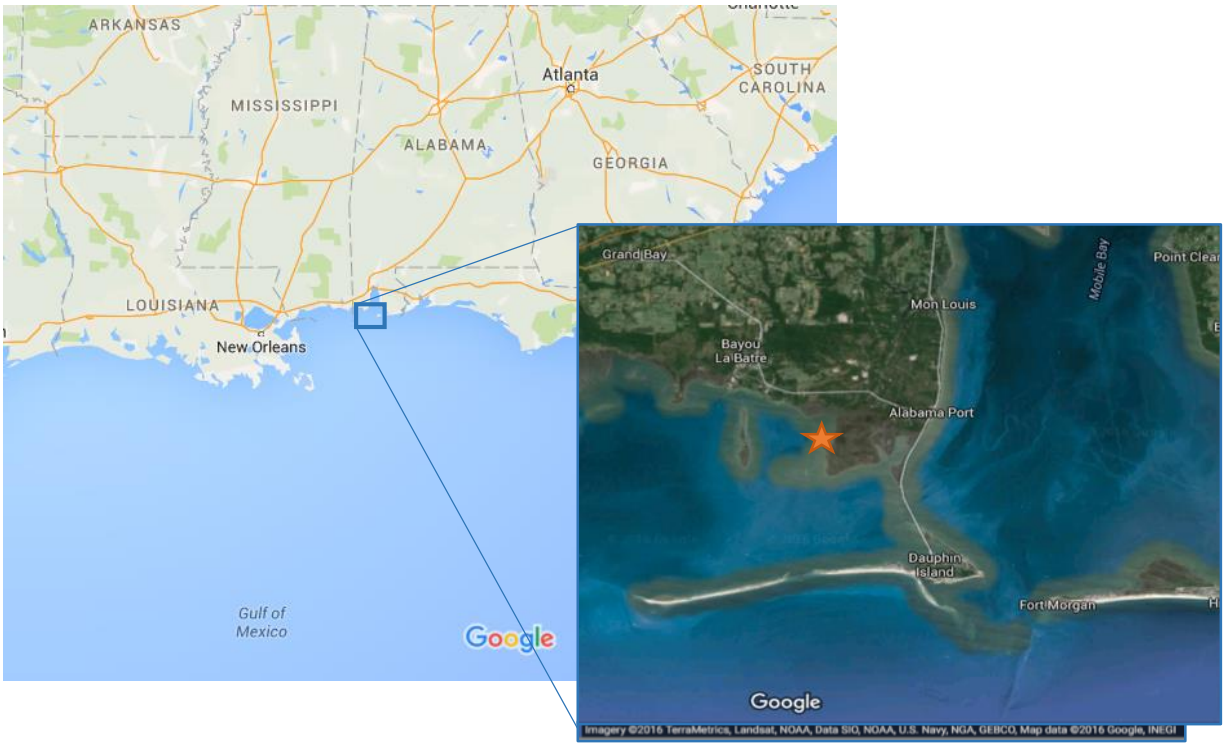

Figure 3.1 Location of the Auburn University Oyster Research and Demonstration Farm in Portersville Bay, Alabama ((AUORDF marked by orange star) (Google Maps, 2016).

The AUORDF was established in 2011 in order to facilitate the development of an off-bottom oyster industry within the state of Alabama, serving as a field research site, juvenile shellfish nursery, training area for shellfish farmers, and an enterprise zone for start-up shellfish farmers. AUORDF has fulfilled its purpose through enabling the development of a competitive off-bottom oyster industry in coastal Alabama. AUSL maintains an 8-acre section of the lease, with space dedicated to conducting research on various oyster culture gear, grow out techniques, and best management practices, with the remainder of the lease dedicated to training and development for beginner oyster farmers.

3.2.2 Gear Type: Floating Cage System

The OysterGro™ is the sole grow out equipment used throughout this project. Developed in Bouctouche, New Brunswick, Canada, the OysterGro™ is an FFCS that was initially adopted by commercial oyster farmers in Atlantic Canada, and has since become a common production method throughout North America's cultured oyster industry (OysterGro™ Company, 2016).

Figure 3.2 The OysterGro™ Flippable Floating Cage System (FFCS) in grow out and desiccation position. (Photo: Walton et al., 2012)

Figure 3.3 The OysterGro™ Flippable Floating Cage System (FFCS) stocked with oysters and deployed at Auburn University Oyster Research and Demonstration Farm (AUORDF).

The FFCS outer housing, interior shelves, and hinged door are constructed out of a 12-gauge vinyl coated wire mesh. Shelves divide the interior into either four or six shelving units, each capable of holding one Vexar® bag. The body of the cage is supported by two air-filled floats or 'pontoons', which allows the cage to float on the water's surface, with oysters exposed to the highly productive photic zone. Farmers are able to suspend the cage into the water column for 'grow out position' or flip the cage onto the pontoons for 'desiccation position' where oysters are suspended above the water for aerial exposure. In addition to keeping the cages on or above the surface, the pontoons can be filled with water and sunk to the seafloor for mitigation against regional climatic conditions (i.e. ice accumulation in the north, hurricanes in the south) (Davis, et al. 2012b; OysterGro™ Company, 2016).

3.2.3 Experimental Design

This project took place over a ~three-month period, from August 25 to November 17, 2015. A fully factorial test of ploidy (2 levels), stocking density (3 levels) and flip regime (2 levels) was developed for this study. The effects of these factors and their interactions were quantified through the response variable of shell dimensions, shell and tissue weight, shell morphology, biofouling, and percent mortality.

The first factor was a comparison between diploid and triploid oysters. Diploid and triploid oysters deployed for this project were entering the final grow out stage, making them ideal subjects for examining quality control techniques during the critical pre-harvest conditioning period. A total of 5400 (2700 diploid, 2700 triploid) oysters were deployed for this study.

The second factor was the stocking density of oysters grown in individual Vexar® bags within the FFCS. Vexar® bags have a recommend stocking density of 150 oysters in the GOM (Davis et al., 2012b). To assess the effect of increasing or decreasing the recommended stocking densities a relatively small amount (~+17%), oysters were deployed at stocking densities of 125, 150, and 175, with three replicates per density (Figure 3.4). Through a randomized block design, oyster bags were assigned to one of the six OysterGro™ cages.

Figure 3.4 Vexar® bags ready for deployment with stocking densities of 175,150, & 125.

The third factor including in this study was the flip regime for oysters grown in the FFCS. Desiccation (flip) is a common quality control method used by farmers to minimize biofouling accumulation on culture gear and individual oysters. Based on work at Auburn University (Davis et al., 2012b), it is recommended that the FFCS be flipped on a weekly regime (7 days) for a duration of ~24 hours (depending on air temperature). Here we tested whether there were differences between a flip regime of weekly and biweekly, holding the desiccation duration constant. Through a randomized block design, three of the cages were assigned to a weekly (24-hour duration) flip regime, and three were randomly assigned to a biweekly (24-hour duration) flip regime.

All oysters used for this study were spawn at the AUSL and were ~6 months old at the time of deployment. Each oyster underwent the same pre-deployment process, consisting of one wash and grading cycle through a QuickTube Sorter™ mechanical rotary style grader manufactured by the Chesapeake Bay Oyster Company (Figure 3.5).

Figure 3.5 A Photo of the QuickTube Sorter™ mechanical rotary style oyster grader used to grade, tumble and wash oysters.

Oysters were processed through the aluminum 'market grading tube', which was manufactured with two hole sizes or 'grades', of 31.75 mm diameter and 44.45 mm diameter, with only the largest grade used for this study (Chesapeake Bay Oyster Company, 2009). The grader was also equipped with a spray wash bar connected to a freshwater supply, providing a steady stream of water for effective cleaning 'tumblewash' of all oysters processed through the grader. Once washed and graded (above 44.45 mm diameter), all oysters were then counted and divided by their respective stocking densities for deployment. Vexar® bags (18 diploids, 18 triploids) were identified with color-coded zip ties representing ploidy and stocking density Individual bags were tagged accordingly and given a randomized placement within the FFCS to ensure unbiased experiment design. On August 25, 2015, tagged bags were brought to AUORDF and deployed into six floating cages (large mesh - six pack model). The cages used throughout this project were previously deployed on the western most run of the AUORDF (Figure 3.6).

Figure 3.6 Photo of the six FFCS (Flippable Floating Cage System) cages used for this study deployed at AUORDF (Auburn University Oyster Research and Demonstration Farm) site in Portersville Bay, Alabama (September 2015).

From August 25 to November 17, (84 days total), weekly trips to the AUORDF were made every Monday and Tuesday morning in order to flip appropriate cages for desiccation (~24-hour duration).

3.2.4 Sampling Protocol

Throughout the study, a destructive sampling process was used to record the response variables of shell height, shell length, shell width, whole wet weight, dry shell weight, dry tissue weight. Samples were taken at ~one-month intervals September 22, October 20, November 17 and consisted of 15 oysters taken out of each bag (36 bags), for a total of 540 oysters being sampled per month. To compensate for the change in stocking density imposed by destructive sampling in September and October, oysters were added to each bag, drawn from a re-stocking population.

Restocking oysters were sorted into eight Vexar® bags (four diploids, four triploids) and deployed into two OysterGro™ cages (large mesh six pack models) within the AUORDF, adjacent to the experimental deployment. Prior to September and October sampling, re-stocking oysters (15 oyster x 18 bags = 270 per ploidy) were collected from the field and brought into AUSL to be desiccated, counted, marked and prepared for restocking protocol.

For record keeping and ease of traceability, each sample was put into a one-gallon Ziploc® freezer bag with individual waterproof tags. All tags included a three-character code to display the ploidy, flip regime and stocking density appointed to the individual sample (i.e. DB1 = diploid, biweekly, 125 density, and TW3 = triploid, weekly, 175 density).

3.2.5 Data Collection

Oysters brought back to the AUSL after each monthly sample (September, October, and November) were frozen to allow processing to occur as time permitted. Processing began with oysters removed from the freezer one bag at a time, where measurements for shell metric data (shell height, shell length, shell width) (Figure 3.7) and whole wet weight (WWW), were recorded before oysters could thaw and prior to destructive sampling. Shell metrics were measured to the nearest 0.01 mm using Mitutoyo IP 67 Electronic Digital calipers (Mitutoyo America, Aurora, IL) (Figure 3.8).

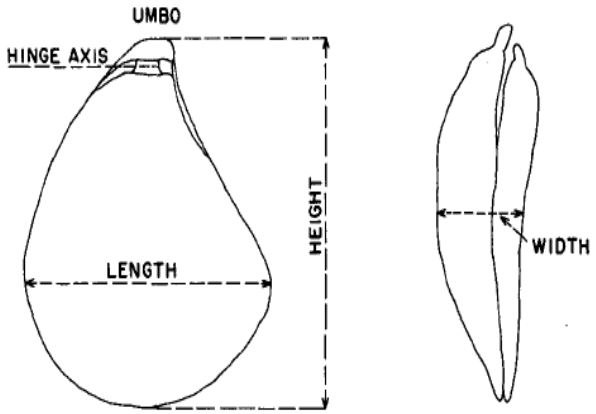

Figure 3.7 Basic shell dimension terminology used for recorded individual oyster shell dimensions of height, length, and width (From Galtsoff, 1964).

Figure 3.8 Oyster measured for shell dimensions (shell height) with digital calipers at Auburn University Shellfish Laboratory (AUSL)

Individual whole wet weight (WWW) of all measured oysters were then weighed to the nearest 0.0001 g using a Mettler Toledo AL204 digital scale (Mettler Toledo, Columbus,

OH). These shell dimensions were used to calculate the cup-shape ration (shell width to shell height), and fan-shape ratio (shell length to shell height) for each sampled oyster, an index that helps define the quality of half-shell oysters (Walton et al., 2013)

Individual oysters that had been measured and weighed were then assigned to numbered Petri dish and laid out on the processing table where they were manually opened (or 'shucked') from the hinge. The meat was removed from the shells and blotted to help remove residual oyster liquor (Figure 3.9). All shells were inspected for any remaining muscle tissue, which was scraped from the adductor muscle using a scalpel.

Figure 3.9 Three oysters being processed for condition index. All oysters are shucked and tissues are separated from the shell, with three aluminum foil boats ready to receive oyster tissue.

Individual oyster meats went into a VWR 6 cm diameter aluminum foil boat (VWR, International) labeled with a matching number of the Petri dish carrying that samples shells. Wet shells were arranged to dry for 48 ± 2 hours at room temperature (20-23°C) while oyster tissue was dried for 48 ± 2 hours at 80°C degrees in a Fisher Scientific® ISOTEMP™ drying oven (Thermo Fisher Scientific Inc., Pittsburgh, PA). The last step of the sampling process was to weigh dried shells and dried tissue to the nearest 0.0001 g using a Mettler Toledo AL204 digital scale (Mettler Toledo, Columbus, OH). Once the dry shell and dry tissue weights were complete, a condition index (CI) was calculated using the following formula from Abbe & Albright (2003):

CI = [dry tissue weight (g) / (whole wet weight (g) – dry shell weight (g))] x 100

In the final sample month (November), 108 oysters (9 per treatment) were randomly selected for a final mean ash-free dry weight (µAFDW) biofouling assessment. Biofouling cleaning consisted of all visible fouling being removed using a 3-inch Russel-Dexter™ oyster shucking knife and steel wire brush. Collected biofouling was assigned to a pre-labeled VWR 6 cm diameter aluminum foil boat (VWR, International), and was carefully placed into a Fisher Scientific® Thermolyne™ Muffle Furnace (Thermo Fisher Scientific Inc., Pittsburgh, PA), set at 500°C for 5 hours. Each biofouling boat was then individually weighed to the nearest 0.0001 g using a Mettler Toledo AL204 digital scale (Mettler Toledo, Columbus, OH).

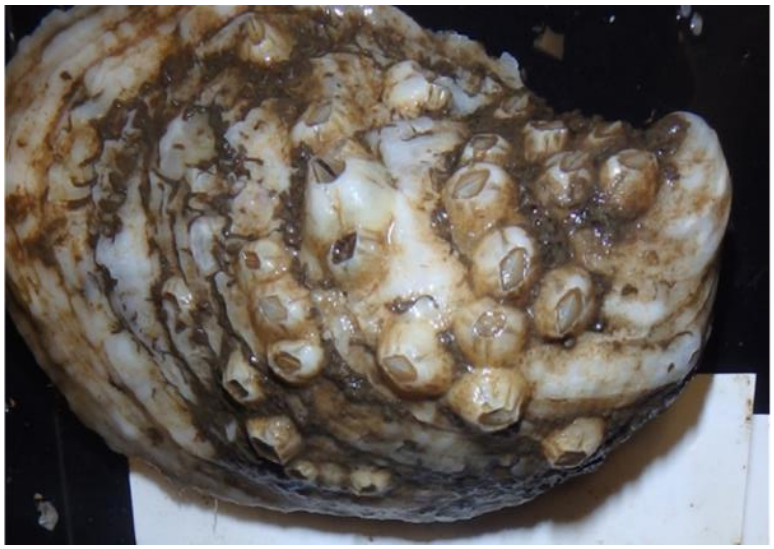

Figure 3.10 A single oyster with substantial barnacle biofouling prior to being processed for a final biofouling assessment (November 2015).

Furthermore, after the final sample period, a total mortality count was done for all oyster treatments. All 36 Vexar® bags were brought back to AUSL, emptied and individually assessed for any dead oysters. Total dead oyster's counts were analyzed across all treatments, allowing for an average mortality rate for each treatment.

3.2.6 Data Analysis

A three-factor test analyzed the effects and interactions of ploidy (diploid, triploid), stocking densities (125, 150, 175) and desiccation regimes (weekly, biweekly), for oysters grown in the FFCS (for a total of 2 x 3 x 2, or 12 treatment combinations). There were three replicates per treatment (12 x 3 = 36 bags total), each sampled over three separate sampling periods.

In order to address any initial (pre-deployment) differences in shell metrics between ploidy (diploid and triploid), a delta value was computed for all measured shell metrics and weight values. Delta values are defined as the difference between an average metric (i.e. shell height, whole wet weight, etc.) for a set of 100 diploid and 100 triploid pre-deployment oysters, to average monthly sampled diploid and triploid oyster metrics. Delta values were used for all response variables except for mean cup shape and mean fan shape as the pre-deploy values were not statistically significantly different from monthly sampled oysters, therefore, delta values were not utilized in statistical analysis for these metrics.

Data were analyzed by month, ploidy, stocking density, and flip and any interaction between them for all measured responsible variables, including; shell dimensions (shell height, shell length, shell width), shell and tissue weight (whole wet weight, dry shell weight, dry tissue weight), shell shape (cup shape, fan shape), condition index ratio and infestation rates of *P. websteri* mud worm (see Manuscript 2). Additionally, in the final sampling month (November), average biofouling and average percent mortality were computed across all treatments.

An ANOVA general linear model was employed to assess any interactions between the four factors (month, ploidy, stocking density, flip), which were recorded for all response variables. Systat® 13 (Systat Software Inc. Chicago, IL) and MiniTab® 17 (State College, PA) statistical software was used to analyze the data. All tests were performed with a significance level of $\alpha = 0.05$ where means were considered significantly different from one another if $p < 0.05$. Where significant interactions were found, a Tukey's post hoc pairwise comparison was performed to further explore results computed by the ANOVA.

3.3 RESULTS

3.3.1 Shell Dimensions

Shell Height

A significant two-way interaction was found between sample date and ploidy on δSH (ANOVA: p = 0.001, Table 3.1). Diploids and triploids did not significantly differ in September or October (p > 0.05), but triploids had significantly higher δSH than diploids in November (triploid = 29.29mm, diploid = 24.21mm) (Figure 3.11; Tukey HSD: p < 0.001 see App. II, Table II.1). There was also a significant effect of flip on δSH, where biweekly oyster mean δSH (18.33 mm) was significantly larger than weekly oyster mean δSH (17.10 mm) (ANOVA: p = 0.015, see App. II, Table II.2). Stocking density had no significant effect on δSH (ANOVA: p = 0.656, Table 3.1).

Table 3.1 ANOVA for delta shell height (δSH).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE DATE	2	5544.24	2772.12	424.15	**0.000**
PLOIDY	1	154.44	154.44	23.63	**0.000**
FLIP	1	40.58	40.58	6.21	**0.015**
STOCKING DENSITY	2	5.55	2.78	0.42	0.656
SAMPLE DATE*PLOIDY	2	109.27	54.63	8.36	**0.001**
SAMPLE DATE*FLIP	2	23.31	11.65	1.78	0.175
SAMPLE DATE*STOCKING DENSITY	4	13.65	3.41	0.52	0.720
PLOIDY*FLIP	1	10.33	10.33	1.58	0.213
PLOIDY*STOCKING DENSITY	2	1.26	0.63	0.10	0.908
FLIP*STOCKING DENSITY	2	2.77	1.38	0.21	0.810
SAMPLE DATE*PLOIDY*FLIP	2	0.81	0.41	0.06	0.940
SAMPLE DATE*PLOIDY*STOCKING DENSITY	4	37.24	9.31	1.42	0.235
SAMPLE DATE*FLIP*STOCKING DENSITY	4	21.19	5.30	0.81	0.523
PLOIDY*FLIP*STOCKING DENSITY	2	23.89	11.94	1.83	0.168
SAMPLE DATE*PLOIDY*FLIP*STOCKING DENSITY	4	47.80	11.95	1.83	0.133
Error	72	470.57	6.54		

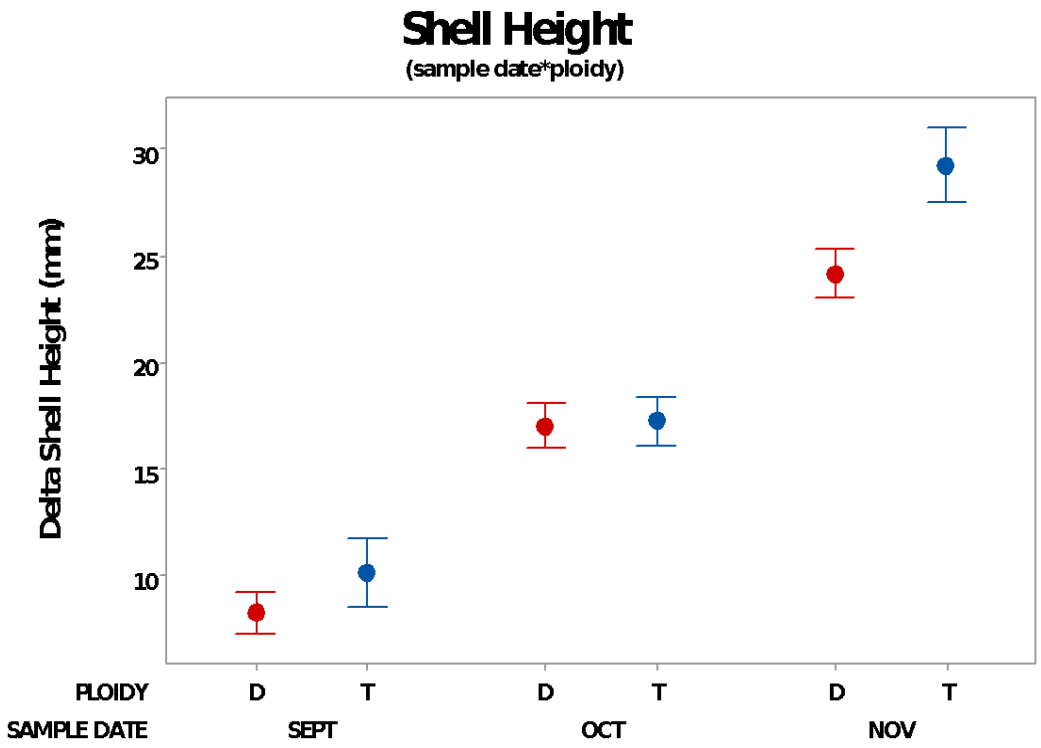

Figure 3.11 The significant effect of sample date and oyster ploidy on delta shell height δSH (mm ± 95%CI), Along the x-axis, D = diploid, T= triploid.

Shell Length

Sample date (ANOVA: $p < 0.001$), ploidy (ANOVA: $p < 0.001$), and flip (ANOVA: $p = 0.006$) had a significant effect on δSL, and no significant interactions were found among these factors (Table 3.2). Oysters underwent significant consecutive growth over the three-month sample period, with mean δSL for September = 6.27mm, October = 11.69mm, and November = 18.39mm (see App. II, Table II.3). Overall, triploid oysters exhibited the largest growth over the course of the study period with triploid oyster mean δSL = 12.62mm and diploid oyster mean δSL = 11.61mm (Figure 3.12; see App. II, Table II.4). Biweekly flipped oysters had significantly larger mean δSL (12.50mm) relative to weekly flipped oyster mean δSL (11.73mm) (Figure 3.13; see App. II, Table II.5) Stocking density had no significant effect on δSL (ANOVA: $p = 0.563$, Table 3.2).

Table 3.2 ANOVA for delta shell length (δSL).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE DATE	2	2657.69	1328.84	672.17	**0.000**
PLOIDY	1	27.43	27.43	13.87	**0.000**
FLIP	1	16.18	16.18	8.18	**0.006**
STOCKDENS	2	2.29	1.14	0.58	0.563
SAMPLE_DATE*PLOIDY	2	11.46	5.73	2.90	0.062
SAMPLE_DATE*FLIP	2	7.87	3.93	1.99	0.144
SAMPLE_DATE*STOCKDENS	4	4.10	1.03	0.52	0.722
PLOIDY*FLIP	1	2.38	2.38	1.20	0.277
PLOIDY*STOCKDENS	2	1.35	0.67	0.34	0.712
FLIP*STOCKDENS	2	5.59	2.79	1.41	0.250
SAMPLE_DATE*PLOIDY*FLIP	2	0.54	0.27	0.14	0.873
SAMPLE_DATE*PLOIDY*STOCKDENS	4	5.50	1.37	0.70	0.598
SAMPLE_DATE*FLIP*STOCKDENS	4	9.63	2.41	1.22	0.311
PLOIDY*FLIP*STOCKDENS	2	1.07	0.54	0.27	0.763
SAMPLE_DATE*PLOIDY*FLIP*STOCKDENS	4	11.48	2.87	1.45	0.226
Error	72	142.34	1.98		

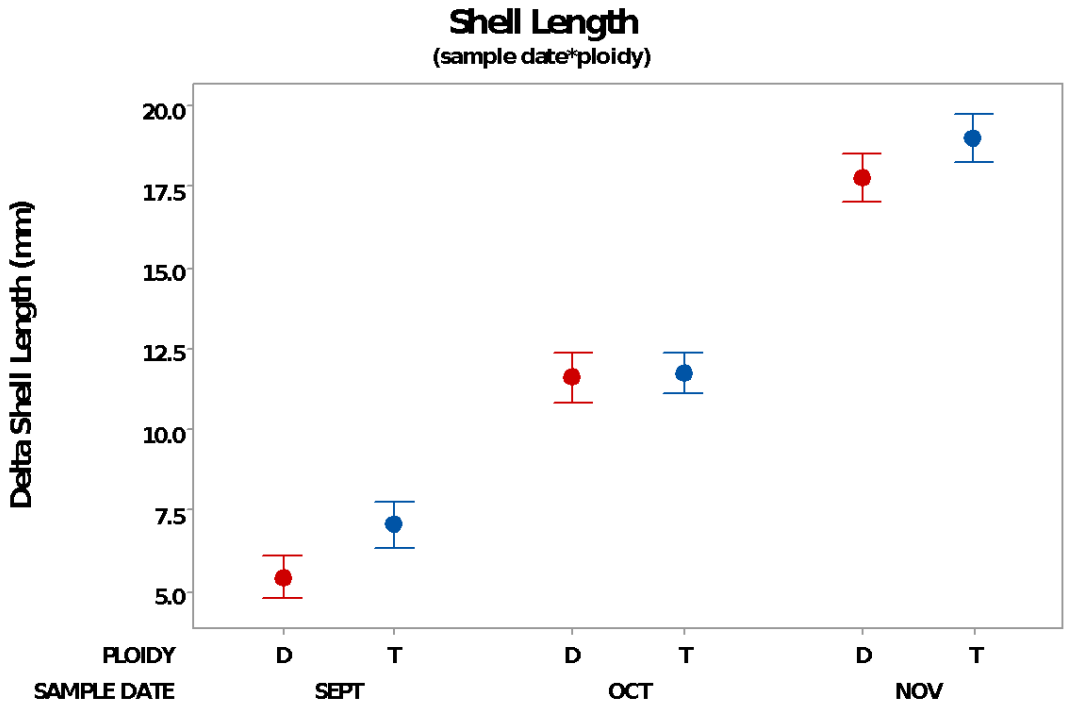

Figure 3.12 The significant effect of sample date and ploidy on delta shell length δSW (mm ± 95%CI). Along the x-axis, D = diploid, T = triploid.

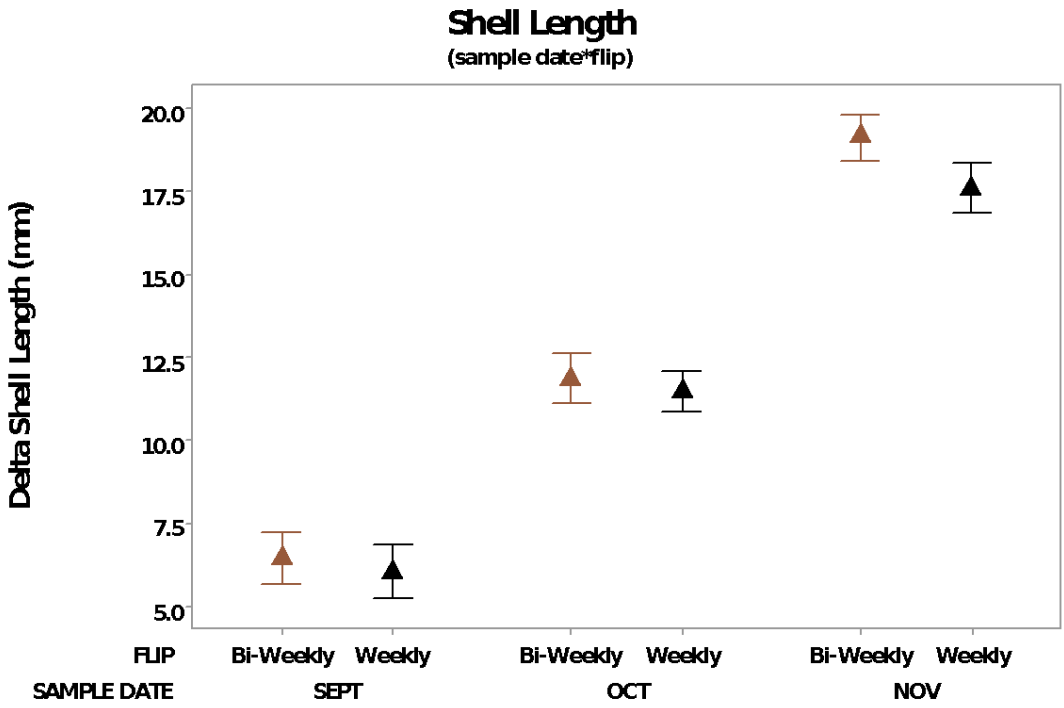

Figure 3.13 The significant effect of sample date and flip frequency on delta shell length δSL (mm ± 95%CI).

Shell Width

A significant four-way interaction was found between sample date, ploidy, flip and stocking density on δSW (ANOVA: p = 0.021, Table 3.3) Interestingly, while there was a four-way interaction amongst all tested factors, there were no significant difference among treatments at any given sample date (Figure 3.14 a, b, c; see App. II, Table II.6). The high level of variation observed, and the high number of pairwise comparisons made, preclude any strong conclusion about the effect of these factors.

Table 3.3 ANOVA for delta shell width (δSW).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE DATE	2	784.361	392.181	655.64	**0.000**
PLOIDY	1	5.401	5.401	9.03	**0.004**
FLIP	1	0.764	0.764	1.28	0.262
STOCKDENS	2	0.284	0.142	0.24	0.789
SAMPLE DATE*PLOIDY	2	3.434	1.717	2.87	0.063
SAMPLE DATE*FLIP	2	1.387	0.694	1.16	0.319
SAMPLE DATE*STOCKDENS	4	2.834	0.708	1.18	0.325
PLOIDY*FLIP	1	0.133	0.133	0.22	0.638
PLOIDY*STOCKDENS	2	1.506	0.753	1.26	0.290
FLIP*STOCKDENS	2	0.662	0.331	0.55	0.578
SAMPLE DATE*PLOIDY*FLIP	2	2.181	1.090	1.82	0.169
SAMPLE DATE*PLOIDY*STOCKDENS	4	2.189	0.547	0.92	0.460
SAMPLE DATE*FLIP*STOCKDENS	4	0.362	0.090	0.15	0.962
PLOIDY*FLIP*STOCKDENS	2	1.348	0.674	1.13	0.330
SAMPLE DATE*PLOIDY*FLIP*STOCKDENS	4	7.398	1.849	3.09	**0.021**
Error	72	43.068	0.598		

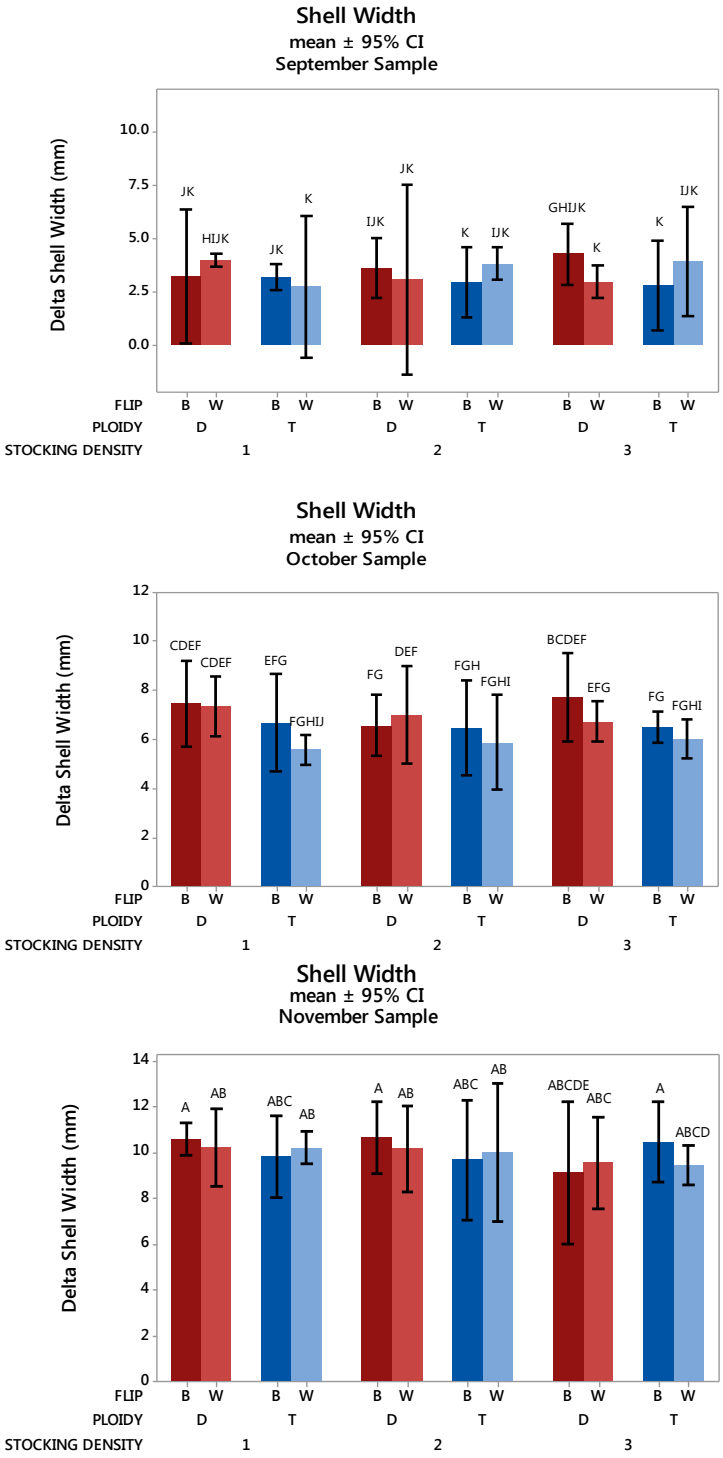

Figure 3.14 a) The significant effect of stocking density, oyster ploidy & flip frequency on mean delta shell width for oysters sampled in September, b) October, c) November. Along the x-axis, 1 = 125 stocking density, 2 = 150, 3 =175, D = diploid, T = triploid, B = biweekly, W = weekly.

3.3.2 Shell and Tissue Weight

Whole Wet Weight

A significant two-way interaction was found between sample date and ploidy on δWWW (ANOVA: $p < 0.001$, Table 3.4). For this interaction, all pairwise comparisons significantly differed ($p < 0.05$), with the exception of the September triploids and October diploids ($p = 0.094$) and October triploids and November diploids ($p = 0.995$) (Figure 3.15; Tukey HSD: $p < 0.001$, see App. II, Table 11.7). There was no significant effect of flip (ANOVA: $p = 0.461$) or stocking density (ANOVA: $p = 0.760$) on δWWW (Table 3.4).

Table 3.4 ANOVA for delta whole wet weight (δWWW).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE DATE	2	25177.9	12588.9	656.45	**0.000**
PLOIDY	1	8818.3	8818.3	459.83	**0.000**
FLIP	1	10.5	10.5	0.55	0.461
STOCKDENS	2	10.6	5.3	0.28	0.760
SAMPLE DATE*PLOIDY	2	2267.3	1133.7	59.11	**0.000**
SAMPLE DATE*FLIP	2	28.2	14.1	0.73	0.483
SAMPLE DATE*STOCKDENS	4	50.7	12.7	0.66	0.622
PLOIDY*FLIP	1	0.4	0.4	0.02	0.888
PLOIDY*STOCKDENS	2	13.3	6.6	0.35	0.709
FLIP*STOCKDENS	2	35.0	17.5	0.91	0.406
SAMPLE DATE*PLOIDY*FLIP	2	21.1	10.5	0.55	0.580
SAMPLE DATE*PLOIDY*STOCKDENS	4	158.8	39.7	2.07	0.094
SAMPLE DATE*FLIP*STOCKDENS	4	54.3	13.6	0.71	0.589
PLOIDY*FLIP*STOCKDENS	2	14.1	7.0	0.37	0.694
SAMPLE DATE*PLOIDY*FLIP*STOCKDENS	4	133.3	33.3	1.74	0.151
Error	72	1380.8	19.2		

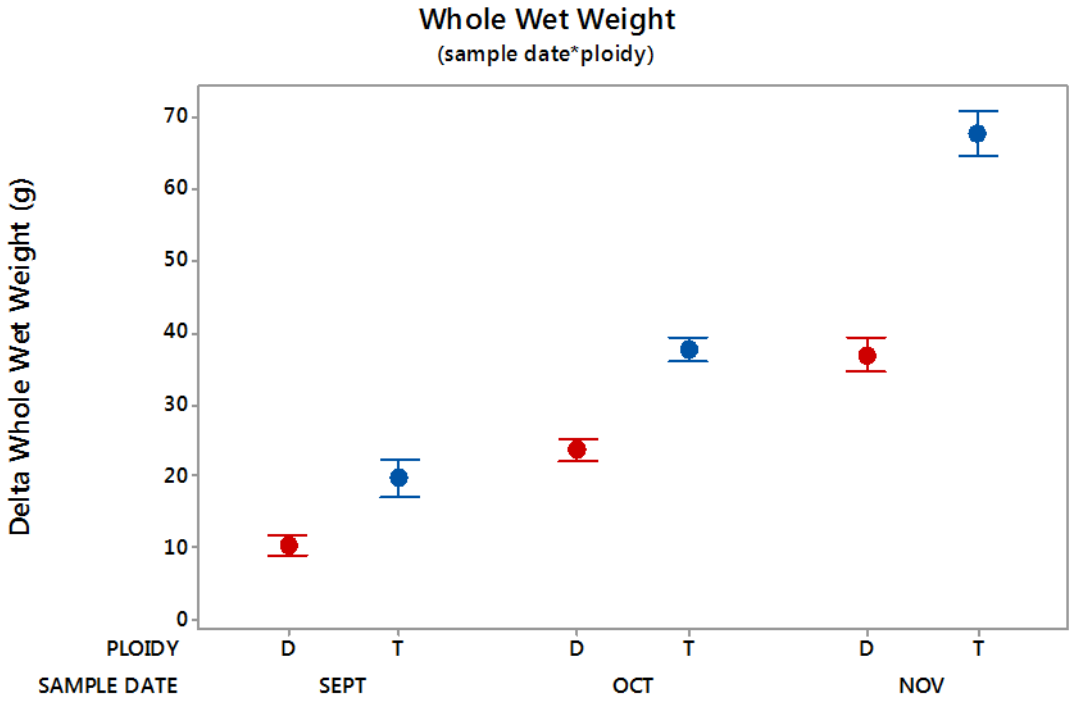

Figure 3.15 The significant effect of sample date and oyster ploidy on delta whole wet weight δWWW (g ± 95% CI). Along the x-axis, D = diploid, T = triploid.

Dry Shell Weight

A significant two-way interaction was found between sample date and ploidy on δDSW (ANOVA: $p < 0.001$, Table 3.5). As with δWWW, for this two-way interaction, all pairwise comparisons significantly differ ($p < 0.05$), with the exception of the September triploids and October diploids ($p = 0.870$) and October triploids and November diploids ($p = 0.117$) (Figure 3.16; Tukey HSD: $p < 0.001$, see App. II, Table II.8). There was no significant effect of flip (ANOVA: $p = 0.165$) or stocking density (ANOVA: $p = 0.798$) on δDSW (Table 3.5).

Table 3.5 ANOVA for delta dry shell weight (δDSW).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE_DATE	2	10395.5	5197.73	561.19	**0.000**
PLOIDY	1	4814.1	4814.13	519.78	**0.000**
FLIP	1	18.2	18.22	1.97	0.165
STOCKDENS	2	4.2	2.10	0.23	0.798
SAMPLE_DATE*PLOIDY	2	1069.0	534.48	57.71	**0.000**
SAMPLE_DATE*FLIP	2	3.9	1.93	0.21	0.812
SAMPLE_DATE*STOCKDENS	4	20.2	5.04	0.54	0.704
PLOIDY*FLIP	1	5.5	5.49	0.59	0.444
PLOIDY*STOCKDENS	2	5.5	2.77	0.30	0.742
FLIP*STOCKDENS	2	21.8	10.88	1.17	0.315
SAMPLE_DATE*PLOIDY*FLIP	2	8.9	4.43	0.48	0.622
SAMPLE_DATE*PLOIDY*STOCKDENS	4	75.9	18.97	2.05	0.097
SAMPLE_DATE*FLIP*STOCKDENS	4	17.8	4.46	0.48	0.749
PLOIDY*FLIP*STOCKDENS	2	9.4	4.72	0.51	0.603
SAMPLE_DATE*PLOIDY*FLIP*STOCKDENS	4	53.4	13.34	1.44	0.230
Error	72	666.9	9.26		

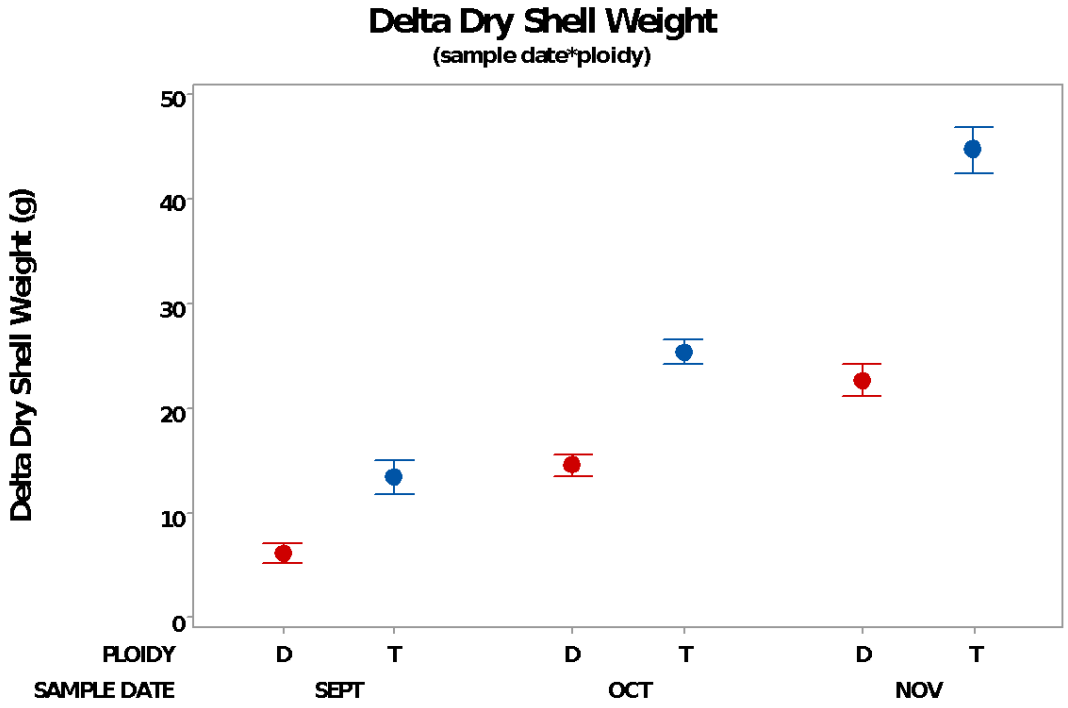

Figure 3.16 The significant effect of sample date and oyster ploidy on delta dry shell weight δDSW (g ± 95% CI). Along the x-axis, D = diploid, T = triploid.

Dry Tissue Weight

A significant three-way interaction was found between sample date, ploidy and flip on δDTW (ANOVA: $p < 0.001$, Table 3.6). Within each sample month, diploid δDTW was always significantly lower than triploid oysters ($p < 0.001$). In each of the first two months, within each ploidy, biweekly and weekly oyster δDTW did not differ significantly ($p < 0.05$). However, in November, triploid biweekly oysters had larger δDTW than triploid weekly oysters ($p < 0.001$), which is opposite to what was seen in diploid oysters, where weekly δDTW was larger than biweekly δDTW ($p = 0.029$) (Figure 3.17). Additionally, δDTW did not differ significantly between September triploids and October diploids and October triploids and November diploids respectively, as was seen in δWWW and δDSW (Tukey HSD: $p > 0.05$, see App.II, Table II.9). There was no significant effect on δDTW from stocking density (ANOVA: $p = 0.065$, Table 3.6).

Table 3.6 ANOVA for delta dry tissue weight (δDTW).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE_DATE	2	54.1156	27.0578	599.64	**0.000**
PLOIDY	1	23.4659	23.4659	520.04	**0.000**
FLIP	1	0.1029	0.1029	2.28	0.135
STOCKDENS	2	0.2561	0.1280	2.84	0.065
SAMPLE_DATE*PLOIDY	2	4.6735	2.3368	51.79	**0.000**
SAMPLE_DATE*FLIP	2	0.9578	0.4789	10.61	**0.000**
SAMPLE_DATE*STOCKDENS	4	0.4185	0.1046	2.32	0.065
PLOIDY*FLIP	1	1.2044	1.2044	26.69	**0.000**
PLOIDY*STOCKDENS	2	0.2291	0.1146	2.54	0.086
FLIP*STOCKDENS	2	0.1385	0.0692	1.53	0.222
SAMPLE_DATE*PLOIDY*FLIP	2	2.8255	1.4127	31.31	**0.000**
SAMPLE_DATE*PLOIDY*STOCKDENS	4	0.2076	0.0519	1.15	0.340
SAMPLE_DATE*FLIP*STOCKDENS	4	0.1534	0.0383	0.85	0.498
PLOIDY*FLIP*STOCKDENS	2	0.1781	0.0890	1.97	0.146
SAMPLE_DATE*PLOIDY*FLIP*STOCKDENS	4	0.3618	0.0905	2.00	0.103
Error	72	3.2489	0.0451		

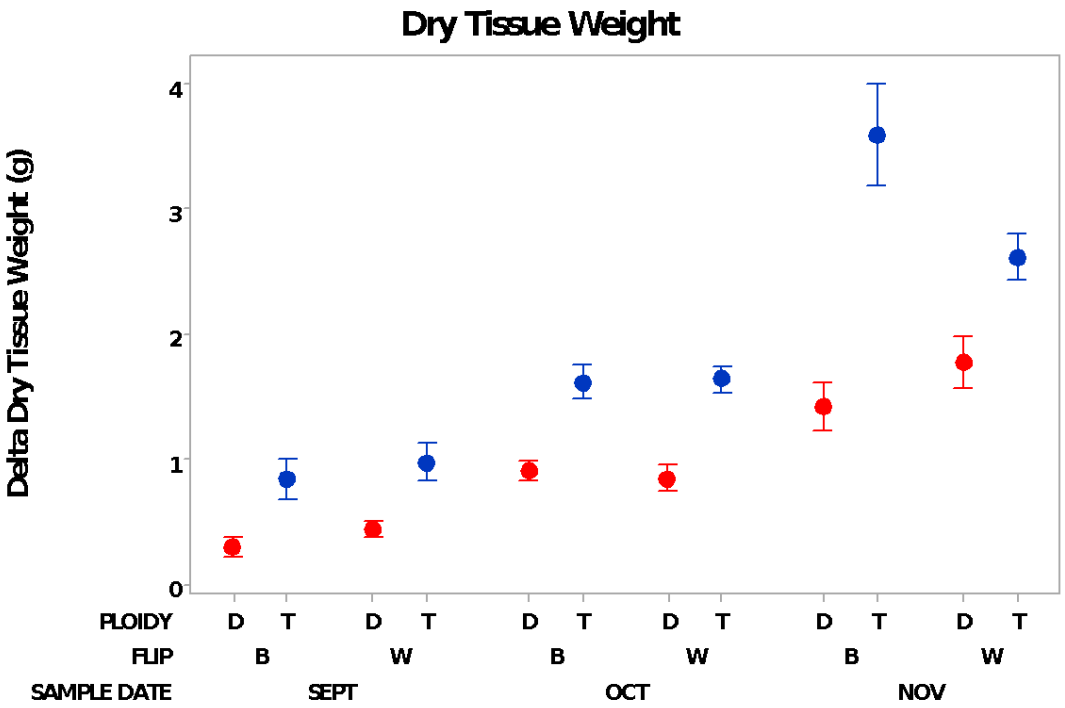

Figure 3.17 The significant effect of sample date, flip frequency and oyster ploidy on delta dry tissue weight δDTW (g ± 95% CI). Along the x-axis, D = diploid, T = triploid, B = biweekly, W = weekly.

3.3.3 Shell Morphology

Cup Shape

A significant two-way interaction was found between sample date and stocking density on µCS (ANOVA: p = 0.044, Table 3.7). Where the only significant difference was that 125 stocking density oysters in October had a significantly larger cup shape compared to 150 stocking density oysters in September (Figure 3.18; Tukey HSD: p = 0.044, see App. II, Table II.10). Additionally, there was a significant effect of ploidy on µCS (ANOVA: p < 0.001, see App. II, Table II.11), where diploid (mean = 0.36) had consistently higher mean µCS over triploid (mean = 0.34) (Figure 3.19). Flip had no significant effect on µCS (ANOVA: p = 0.213, Table 3.7).

Table 3.7 ANOVA for mean cup shape (µCS).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE_DATE	2	0.001461	0.000731	5.74	**0.005**
PLOIDY	1	0.009193	0.009193	72.23	**0.000**
FLIP	1	0.000201	0.000201	1.58	0.213
STOCKDENS	2	0.000271	0.000135	1.06	0.350
SAMPLE_DATE*PLOIDY	2	0.000650	0.000325	2.55	0.085
SAMPLE_DATE*FLIP	2	0.000763	0.000381	3.00	0.056
SAMPLE_DATE*STOCKDENS	4	0.001319	0.000330	2.59	**0.044**
PLOIDY*FLIP	1	0.000359	0.000359	2.82	0.097
PLOIDY*STOCKDENS	2	0.000306	0.000153	1.20	0.307
FLIP*STOCKDENS	2	0.000158	0.000079	0.62	0.541
SAMPLE_DATE*PLOIDY*FLIP	2	0.000404	0.000202	1.59	0.211
SAMPLE_DATE*PLOIDY*STOCKDENS	4	0.000032	0.000008	0.06	0.993
SAMPLE_DATE*FLIP*STOCKDENS	4	0.000301	0.000075	0.59	0.670
PLOIDY*FLIP*STOCKDENS	2	0.000474	0.000237	1.86	0.163
SAMPLE_DATE*PLOIDY*FLIP*STOCKDENS	4	0.000822	0.000205	1.61	0.180
Error	72	0.009163	0.000127		

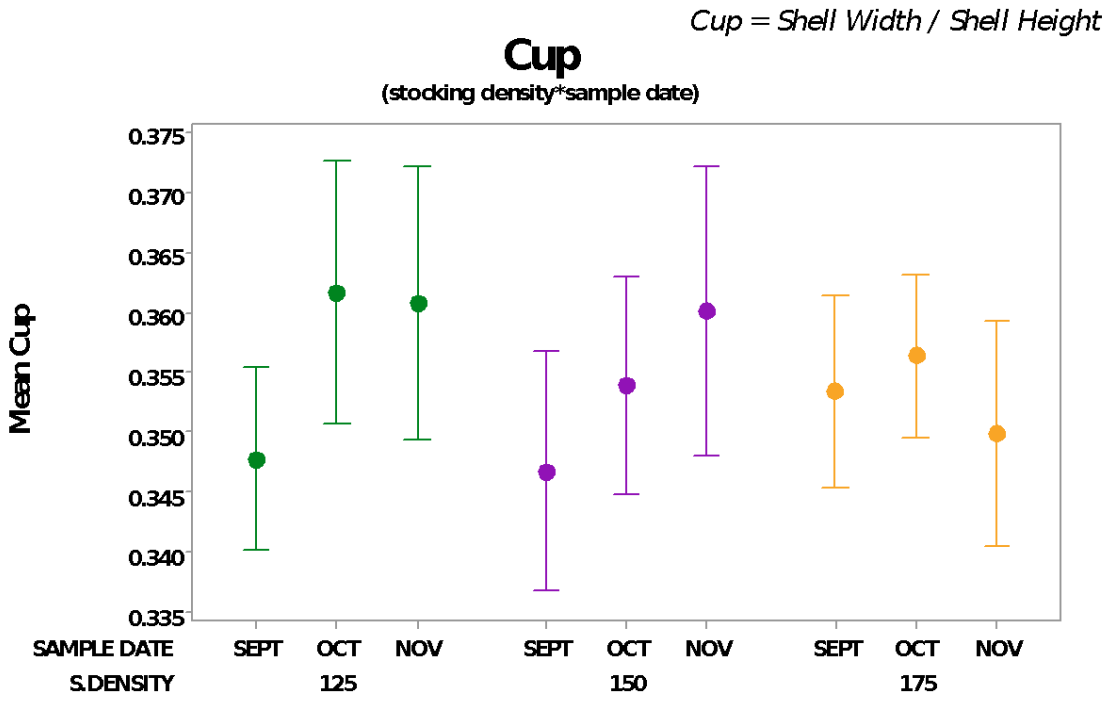

Figure 3.18 The significant effect of stocking density and sample date on mean cup shape (µCS).

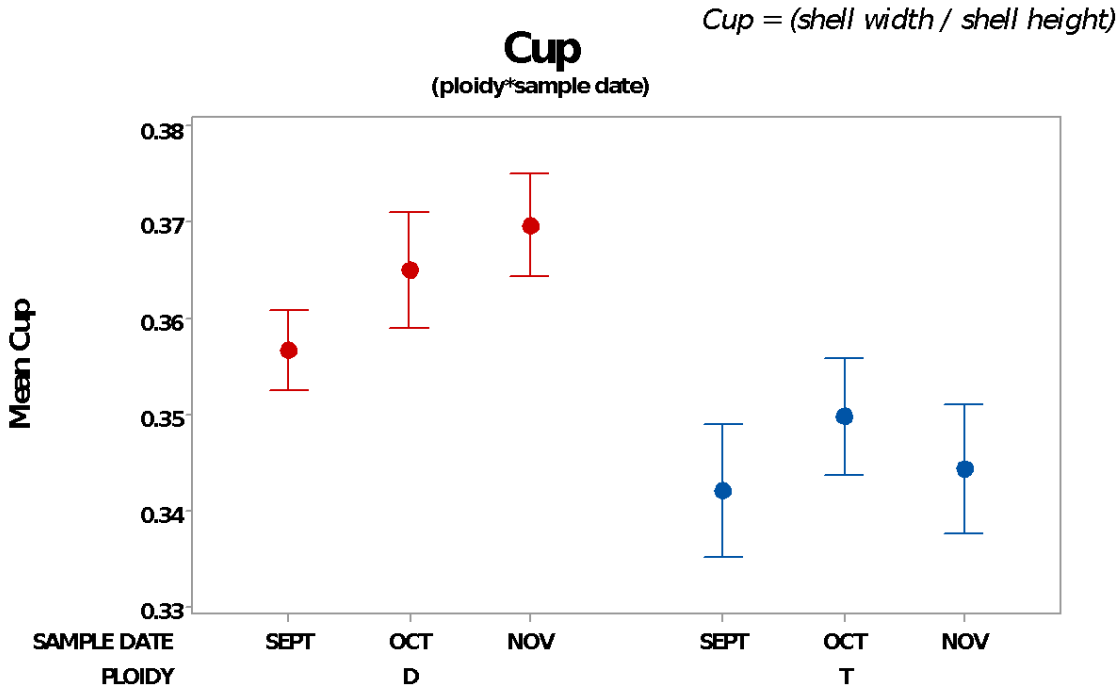

Figure 3.19 The significant effect of ploidy on mean cup shape (µCS) visualized for all three sample dates. Along the x-axis, D = diploid, T = triploid.

Fan Shape

A significant two-way interaction was found between sample date and ploidy on µFS (ANOVA: p = 0.001, Table 3.8). While µFS between ploidy did not differ significantly within the months of September and October (p > 0.05), in November triploids had significantly lower µFS than diploids (Figure. 3.20; Tukey HSD: p < 0.001, App. II, Table II.12) There was no significant effect of flip (ANOVA: p = 0.994) or stocking density (ANOVA: p = 0.758) on µFS.

Table 3.8 ANOVA for mean fan shape (µFS).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE_DATE	2	0.000115	0.000058	0.16	0.854
PLOIDY	1	0.009177	0.009177	25.18	**0.000**
FLIP	1	0.000000	0.000000	0.00	0.994
STOCKDENS	2	0.000203	0.000101	0.28	0.758
SAMPLE_DATE*PLOIDY	2	0.005271	0.002635	7.23	**0.001**
SAMPLE_DATE*FLIP	2	0.000130	0.000065	0.18	0.837
SAMPLE_DATE*STOCKDENS	4	0.000798	0.000199	0.55	0.702
PLOIDY*FLIP	1	0.000046	0.000046	0.13	0.724
PLOIDY*STOCKDENS	2	0.000037	0.000018	0.05	0.951
FLIP*STOCKDENS	2	0.001756	0.000878	2.41	0.097
SAMPLE_DATE*PLOIDY*FLIP	2	0.000113	0.000057	0.16	0.857
SAMPLE_DATE*PLOIDY*STOCKDENS	4	0.000835	0.000209	0.57	0.683
SAMPLE_DATE*FLIP*STOCKDENS	4	0.001639	0.000410	1.12	0.352
PLOIDY*FLIP*STOCKDENS	2	0.000758	0.000379	1.04	0.359
SAMPLE_DATE*PLOIDY*FLIP*STOCKDENS	4	0.001733	0.000433	1.19	0.323
Error	72	0.026237	0.000364		

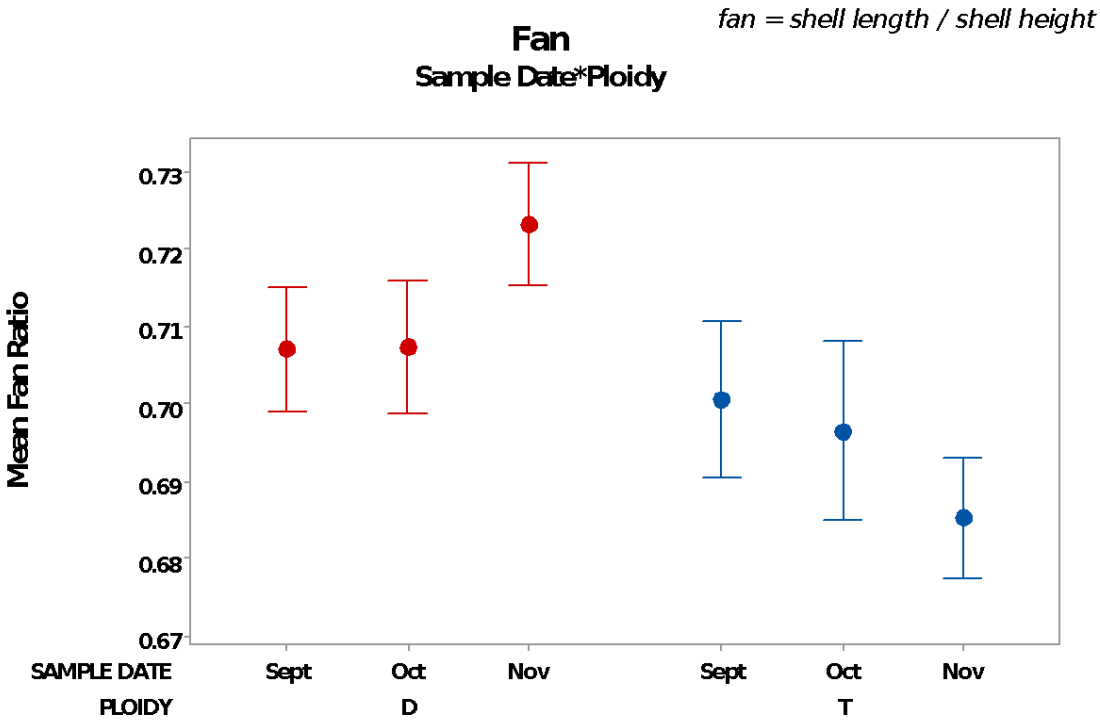

Figure 3.20 The significant effect of sample date and oyster ploidy on mean fan shape (μFS). Along the x-axis, D = diploid, T = triploid.

3.3.4 Condition Index

A significant three-way interaction was found among sample date, flip and ploidy on a change in δCI (refer to section 3.2.5 for formula) (ANOVA: $p < 0.001$, Table 3.9). For the first two sampling months (September, October), triploid oysters had significantly higher mean δCI relative to diploid oysters regardless of the flip regime (Figure 3.21; Tukey HSD, $p < 0.05$, see App. II, Table II.13). Interestingly, in the month of November triploid biweekly mean δCI no longer differed from diploid weekly flipped oyster mean δCI (Tukey HSD: $p = 0.069$, App. II, Table II.13). Additionally, during this same month, triploid weekly flipped oyster mean δCI no longer significantly differed from both diploid weekly and biweekly flipped oysters mean δCI (Tukey HSD: $p = 0.993$, $p = 0.368$, App. II, Table II.13). The highest mean δCI value for diploid was found in November weekly flipped oysters (high = 13.79), with the lowest mean δCI in September biweekly flipped oysters (mean = 6.24) (see App I. Table 1.1 & 1.3). The highest mean-δCI value for triploids were found in September weekly flipped oysters (mean = 17.920), while the lowest mean δCI found in November weekly (mean = 12.127) (see App I, Table 1.4 & 1.6).

Additionally, a significant two-way interaction between ploidy and stocking density had an effect on changes in condition index δCI (ANOVA: $p = 0.010$, Table 3.9). Triploid oysters had significantly higher δCI values when compared to diploids over all three stocking density regimes (Figure 3.22; Tukey HSD: $p < 0.001$, App. II, Table II.14). While mean δCI did not significantly differ across the three stocking densities for diploids ($p > 0.05$), there were differences among stocking densities for triploids. Triploid at 125 stocking density had significantly higher mean δCI than triploids at 175 stocking density (Tukey HSD: $p = 0.033$, see App. II, Table II.14).

Table 3.9 ANOVA for mean delta condition index (δCI).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE_DATE	2	15.84	7.919	2.73	0.072
PLOIDY	1	435.27	435.270	149.92	**0.000**
FLIP	1	10.28	10.281	3.54	0.064
STOCKDENS	2	7.63	3.816	1.31	0.275
SAMPLE_DATE*PLOIDY	2	67.35	33.677	11.60	**0.000**
SAMPLE_DATE*FLIP	2	21.42	10.709	3.69	**0.030**
SAMPLE_DATE*STOCKDENS	4	23.10	5.775	1.99	0.105
PLOIDY*FLIP	1	29.61	29.612	10.20	**0.002**
PLOIDY*STOCKDENS	2	28.50	14.248	4.91	**0.010**
FLIP*STOCKDENS	2	0.41	0.205	0.07	0.932
SAMPLE_DATE*PLOIDY*FLIP	2	84.31	42.153	14.52	**0.000**
SAMPLE_DATE*PLOIDY*STOCKDENS	4	27.74	6.935	2.39	0.059
SAMPLE_DATE*FLIP*STOCKDENS	4	34.63	8.657	2.98	0.025
PLOIDY*FLIP*STOCKDENS	2	7.33	3.666	1.26	0.289
SAMPLE_DATE*PLOIDY*FLIP*STOCKDENS	4	2.57	0.642	0.22	0.92
Error	72	209.04	2.903		

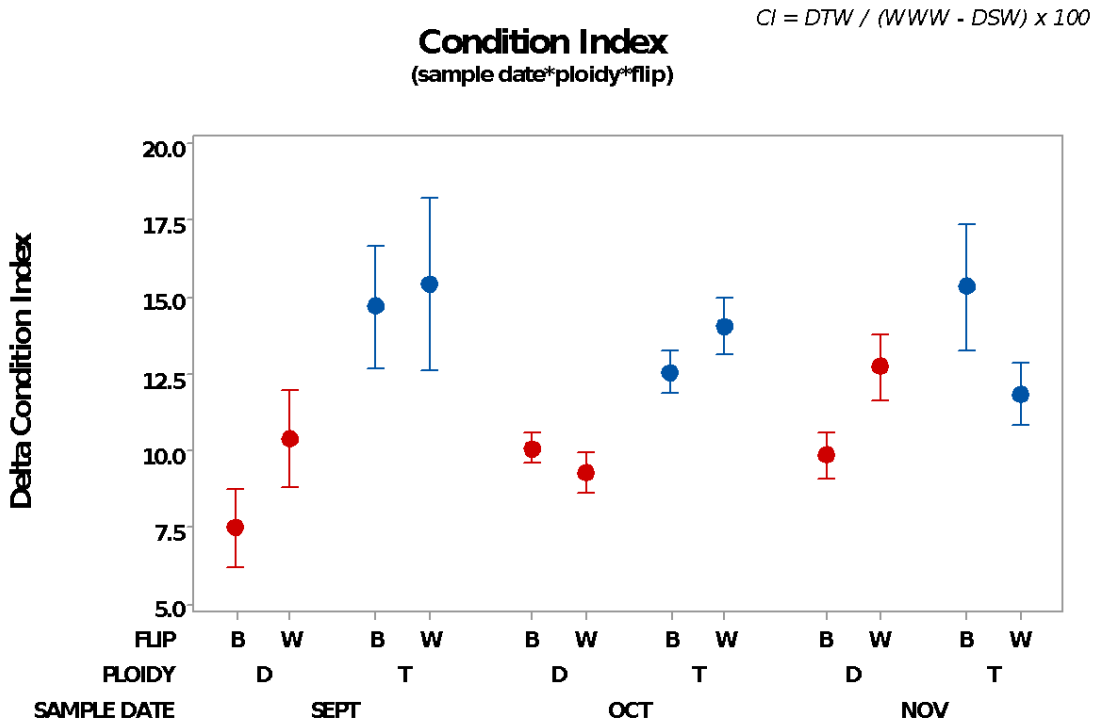

Figure 3.21 The significant effect of sample date, oyster ploidy and flip frequency on mean delta condition index δCI. Along the x-axis, D = diploid, T = triploid, B = biweekly, W = weekly.

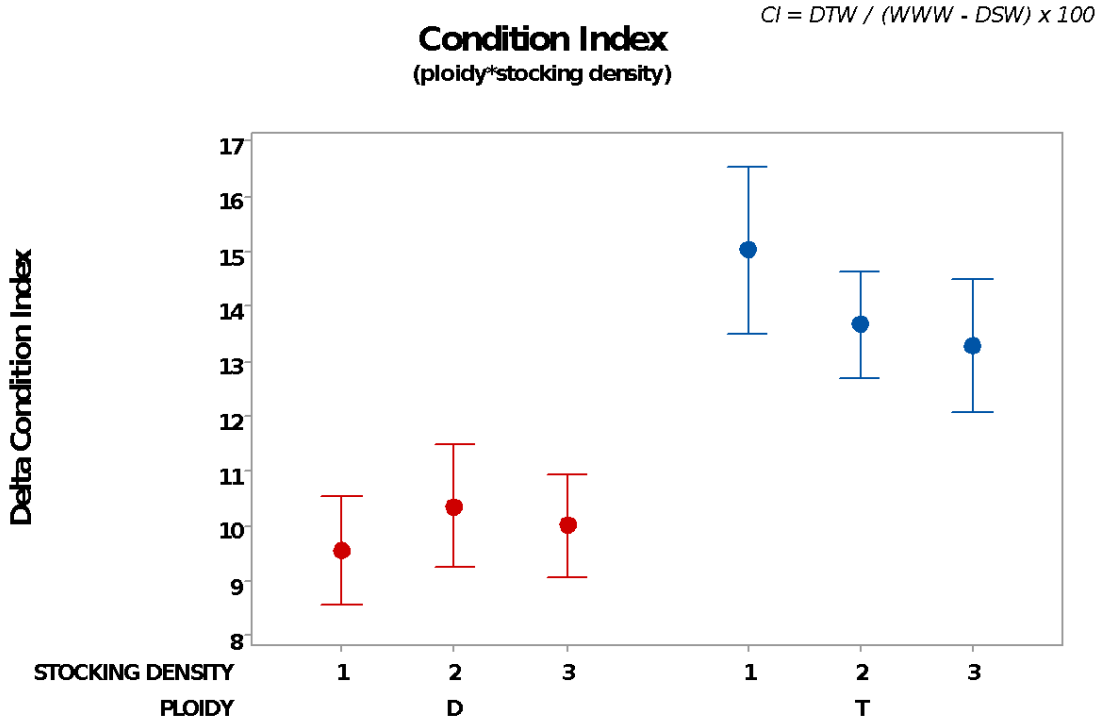

Figure 3.22 The significant effect of oyster ploidy and stocking density on mean delta condition index δCI. Along the x-axis, 1 = 125 stocking density, 2 = 150, 3 =175, D = diploid, T = triploid.

3.3.5 Biofouling

There was a significant two-way interaction found between flip and stocking density on µAFDW of biofouling organisms (ANOVA: p = 0.002, Table 3.10). The lowest µAFDWs were found in the weekly flipped oysters, where µAFDW did not differ amongst stocking densities (Figure 3.23; Tukey HSD: p = 1.000, see App. II, Table II.15). The highest µAFDW was found in biweekly flipped oysters at 125 stocking density, which significantly differed from the other two biweekly stocking densities (Tukey HSD: p < 0.05, see App. II, Table II.15). Biweekly 150 and 175 stocking densities did not significantly differ from one another, however 150 stocking density was found to have the lowest µAFDW (Tukey HSD: p = 0.687, see App. II, Table II.15) There was no significant effect of ploidy on µAFDW (ANOVA: p = 0.779, Table 3.10).

Table 3.10 ANOVA for Mean Ash Free Dry Weight (µAFDW).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ploidy	1	0.000114	0.000114	0.08	0.779
Flip	1	0.063977	0.063977	44.41	**0.000**
Stock Density	2	0.018169	0.009084	6.31	**0.003**
Ploidy*Flip	1	0.000003	0.000003	0.00	0.963
Ploidy*Stock Density	2	0.004302	0.002151	1.49	0.230
Flip*Stock Density	2	0.019076	0.009538	6.62	**0.002**
Ploidy*Flip*Stock Density	2	0.003801	0.001900	1.32	0.272
Error	96	0.138299	0.001441		

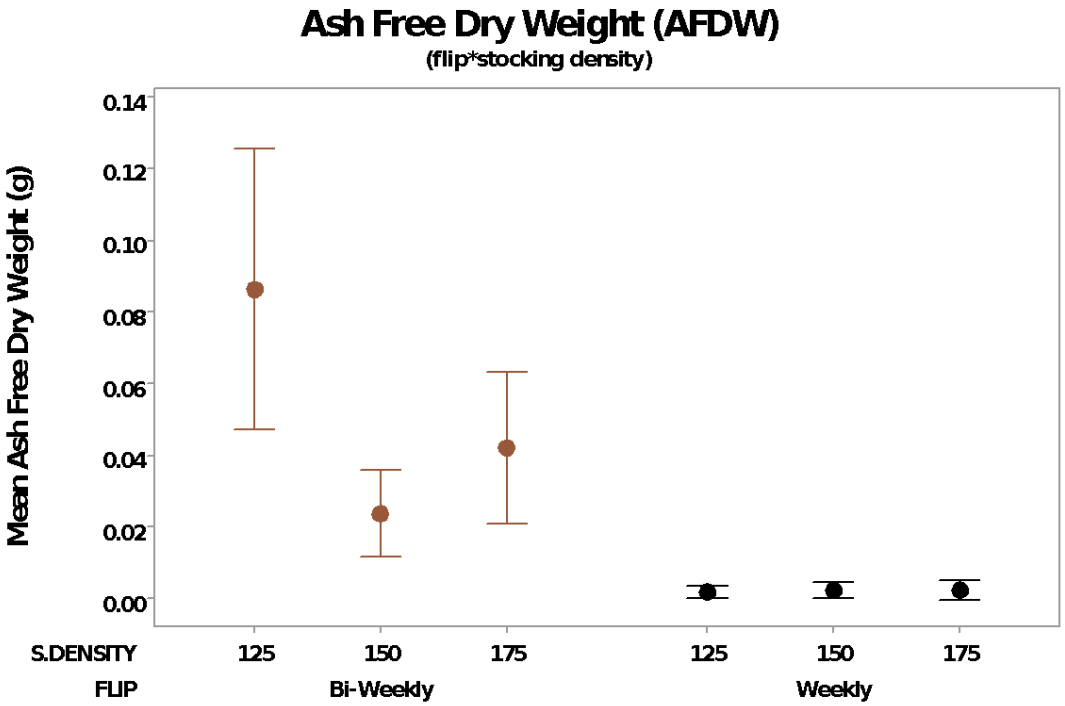

Figure 3.23 The significant effect of flip frequency and stocking density on mean ash free dry weight µAFDW (g±95% CI).

3.3.6 Percent Mortality

There was a significant effect of ploidy (ANOVA: $p < 0.001$, Table 3.11) found on mean percent mortality µPM, where diploid oysters had higher µPM (mean diploid = 6.9 %, mean triploid = 2.8 %, see App.II, Table II.16, Figure 3.24). There was no significant effect of flip or stocking density on µPM (ANOVA: $p = 0.543$, $p = 0.385$, Table 3.11).

Table 3.11 ANOVA for Total Percent Mortality (µPM).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ploidy	1	0.014954	0.014954	16.93	**0.000**
Flip	1	0.000337	0.000337	0.38	0.543
StockDen	2	0.001753	0.000877	0.99	0.385
Ploidy*Flip	1	0.001620	0.001620	1.83	0.188
Ploidy*StockDen	2	0.000734	0.000367	0.42	0.665
Flip*StockDen	2	0.002817	0.001408	1.59	0.224
Ploidy*Flip*StockDen	2	0.001059	0.000530	0.60	0.557
Error	24	0.021202	0.000883		

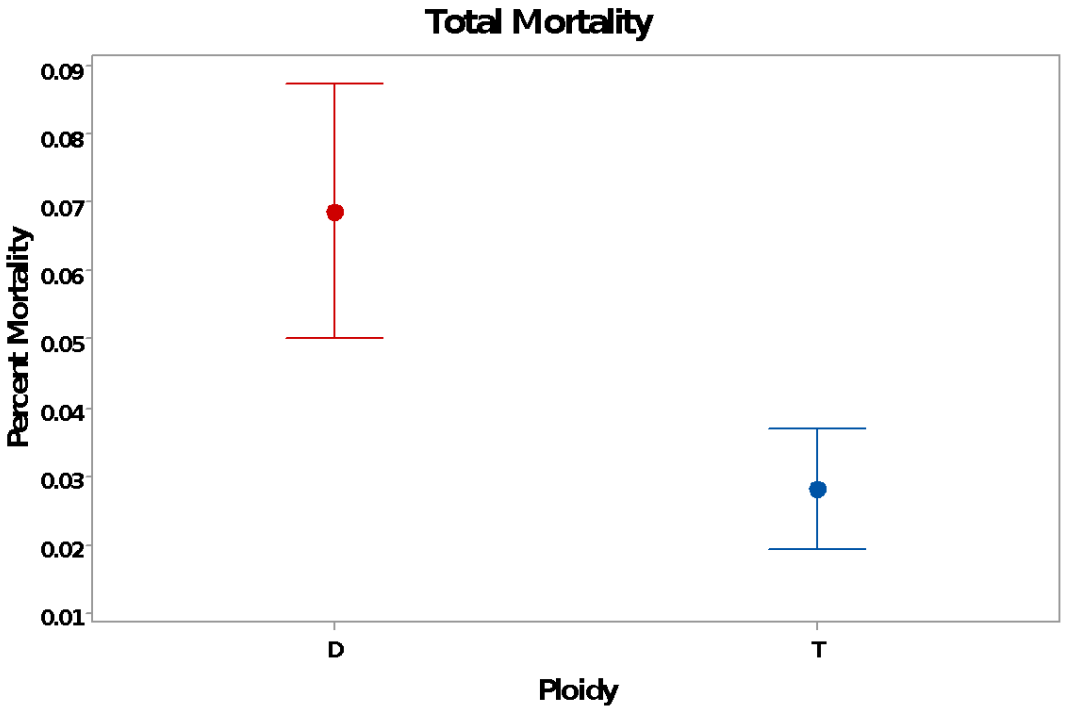

Figure 3.24 The significant effect of ploidy on total percent mortality µPM. Along the x-axis D = diploid, T = triploid.

3.4 Discussion

The oysters deployed for this study were entering the final grow out phase before market size, making them ideal subjects for quality control techniques and conditioning during the critical pre-harvest conditioning period. Here we consider the following five categories of response.

1) *Shell Dimensions* includes the metrics collected for delta shell height (δSH), delta shell length (δSL) and delta shell width (δSW)
2) *Shell and Tissue Weights* include the weight variables collected for delta whole wet weight (δWWW), delta dry shell weight (δDSW), and delta dry tissue weight (δDTW)
3) *Shell Morphology* includes the metrics of mean cup shape (μCS), mean fan shape (μFS), and Delta Condition Index (δCI)
4) *Biofouling* includes the final month (November) values collected for mean ash-free dry weight biofouling (μAFDW)
5) *Percent Mortality* includes the final month (November) values collected for mean percent mortality (μPM) across all treatments

The following discussion explores the effect and interactions of ploidy, stocking density and flip regime on each category of response. Not surprisingly, many results within and across each category are correlated, and not independent of one another.

3.4.1 Shell Dimensions

As the oysters were growing over the duration of this study, it was anticipated that sample date would have a significant effect on all shell dimensions as seen in both a single effect (δSL) and interactive effect (δSH and δSW). More surprisingly, given prior work that has demonstrated the effect of stocking density on shell growth (Honkoop & Bayne, 2002; Comeau et al., 2011; Davis, 2013), in this study, stocking density only had an interactive effect on δSW (described below), but not δSL or δSH. This was perhaps because relatively minor changes in stocking density were tested ($\pm$ 17%). Additionally, this test was conducted in the final phase of grow-out and not over the early life cycle.

In addition, both ploidy and flip had an effect on all shell dimensions measured throughout this study. While both triploid and diploid oysters showed increases, triploids

achieved significantly higher δSH by November (final sample) (triploid mean δSH = 29.29mm, diploid mean δSH = 24.22mm) and had consistently higher mean δSL across the study, validating their ability to grow larger and often faster than diploid oyster (Nell, 2002) (Tukey HSD: $p < 0.001$, see App. II, Table II.1) (ANOVA: $p = 0.001$, Table 3.1, section 3.3.1, see Figure 3.11, 3.12).

Interestingly, regardless of ploidy, all biweekly flipped oysters had larger δSH and δSL than weekly flipped oysters, where again, the highest average values were measured in November (mean δSH = 28.02, mean δSL = 19.16). The overall increase in growth in these two metrics demonstrated by the biweekly flipped oysters could be explained by at least two scenarios. First, biweekly treatments provided one additional day of submersion over each two week period, relative to the weekly treatments (and typically two full days over each monthly sampling period). For an oyster to feed and grow, it must be submerged within the water column, therefore, one could expect the longer submersion growth period would result in larger growth. Second, the act of flipping the oysters may break off new shell growth. The doubling in flipping frequency in the weekly treatment may have led to lower δSH and δSL values. Of course, these alternatives are not exclusive to each other, and may have been occurring simultaneously. Further experimentation would be required to distinguish these hypotheses.

As noted above, a significant four-way interaction found between sample date, ploidy, flip and stocking density on δSW, however, at any given sample month, there were no significant differences among treatment, indicating that sample date played an important role within this interaction. A lacking triploid dominance was found in δSW, which could be explained by the tendency for triploid oysters to excel in horizontal growth (δSH and δSL), with less energy directed towards vertical growth (δSW), which has been suggested in by

Another explanation could be the tendency for an oyster's vertical growth or 'cup depth' (δSW) to grow much slower than horizontal growth δSH, δSL. An implication of this for triploid oysters especially is that a relatively decreased rate in dSW growth to dSH and dSL reduces the optimal cup shape in these oysters. Furthermore, the effect of flip on δSW may not be as pronounced because of slower growth rates associated with δSW. Because of a difference in timescales, a weekly versus biweekly flip regime may not impact growth rates of dSW as substantially. It is important to point out that shell width δSW is directly related to the formation of a deep cup, a trait that is highly coveted in the half-shell oyster industry. While it

was difficult to identify any clear trends within the δSW four-way interaction, it was clear that the tested factors are influencing even the most basic shell dimensions. Overall, it appears as though both ploidy and flip regime have the largest effect on oyster shell growth and shell dimensions (δSH, δSL, and δSW).

3.4.2 Shell and Tissue Weights

The weight variables measured in this study were whole wet weight, dry shell weight and dry tissue weight (δWWW, δDSW, δDTW). These values provided a comprehensive understanding of oyster weight variability and were used to calculate oyster condition index δCI. It is important to note that δWWW, δDSW, and δDTW are correlated metrics and are not independent of one another, in particular, δWWW is composed largely of δDSW and these would be expected to be highly correlated. However, the individual metrics are important components of CI ratio, which merits their individual analysis. As with shell dimensions, it was expected that sample date would have a significant effect on all weight variables. Sample date and ploidy together had a significant effect on δWWW and δDSW, whereas, δDTW was impacted by a significant three-way interaction between sample date, ploidy and flip.

Triploid oyster weight metrics were found to be consistently significantly higher than diploid oyster weights (shell & tissue) throughout the study (Tukey HSD: $p < 0.001$, see App. II, Table II.7, II.8. II.9). In fact, average diploid weight values for δWWW and δDSW were offset by approximately one month when compared to triploid weight values. For example, triploid δWWW in the month of September (mean = 19.78) did not significantly differ from diploid δWWW in the month of October (mean = 23.67) (see App II, Table II.7). This same offset was found for δDSW as well. Again, the fast growth in triploid oysters is both apparent in shell dimension as well as weight values. There was no significant effect of flip or stocking density on δWWW and δDSW (section 3.3.2, see Table 3.4, 3.5).

For δDTW, there was a significant three-way interaction among sample date, ploidy and flip regime, although again no effect of stocking density. As with δWWW and δDSW, triploids were heavier than diploids at each time point. By November, however, flip regime became apparent where biweekly flipped triploid oysters had significantly higher average δDTW than weekly flipped triploid oysters (section 3.3.2, see Figure 3.17; Tukey HSD: $p < 0.001$, see App. II, Table II.9) The opposite holds true for diploid oysters here, where weekly flipped oysters have significantly higher average δDTW when compared to biweekly oysters

(Tukey HSD: p = 0.029, see App. II, Table II.9). Although significant, the differences found between diploid biweekly (δDTW=1.412g) and weekly (δDTW=1.769g) in the month of November are not as substantial as those found for triploid oysters in the same month (biweekly δDTW = 3.588g, weekly δDTW = 2.609g) (see App. II, Table II.9)

For triploid oysters, it is conceivable that the longer the oysters are submerged, (i.e. biweekly flipped oysters) the longer they have to feed, effectively increasing tissue growth. The peak in δDTW for triploids recorded in November may have been triggered by an increase in food abundance, although this was not measured in this study, so further investigation would be required to address this hypothesis. Alternatively, a change in the season marked by slight decreases in solar insolation over the study period may suggest that cooling temperatures could decrease metabolic rates as oysters prepare for cooler winter months, thereby increasing growth (i.e. δDTW). While environmental metrics are not considered in the scope of this study, it is conceivable that these various regional factors could influence shell and tissue growth.

What remains unclear was why this does not hold true for diploid biweekly flipped oysters. One possibility to explain the trend seen in diploid oysters could be linked to their reproductive cycle, which typically occurs over the warm summer months. During reproduction, growth in δDTW could decline accompanied by an increase in gamete production (Gagnarie et al., 2006). Conceivably, weekly flipped diploids may have spawned earlier, which could allow δDTW to recover to higher values than seen in biweekly flipped diploids by the month of November. Perhaps, the weekly 24-hour exposure to warm air temperature and direct sunlight could have triggered diploid weekly oysters to spawn earlier or more often than biweekly flipped oysters.

3.4.3 Shell Morphology

Shell morphology includes the mean cup shape and mean fan shape. Within the half-shell oyster industry, cup and fan values are used as a ranking system for high-quality oysters (typically only qualitatively), yet specifically, cup shape of larger than 0.25 and a fan shape larger than 0.63 are considered to be defining qualities of high-quality oysters (Brake et al. 2003; Cheney, 2010).

For cup shape, stocking density did play a role in the results; a significant interactive effect of sample date and stocking density was found on μCS. Although, there was significant

variation in the results only one significant pairwise difference was observed between September 150 stocking density (μCS = 0.347) and October 125 stocking density (μCS = 0.362) (Tukey HSD: $p = 0.044$, see App. II, Table II.10). Looking at the trends, cup appeared to generally increase over time in the two lowest stocking densities but appeared to not change in the highest stocking density (section 3.3.3, see Figure 3.18).

There was a significant effect of ploidy on mean μCS throughout this study, where diploid oysters had consistently higher μCS at all three sample periods (section 3.3.3, see Figure 3.19). An explanation for the lower mean μCS found in triploid oysters was likely due to a faster growth of δSH relative to δSW over the three-month period. Overall, diploid oyster μCS ranged from 0.349 to 0.378, whereas, triploid oyster μCS ranged from 0.331 to 0.360 (see App. I, Table 1.1 – 1.6). While the ranges overlapped, and both diploid and triploid μCS were well above the recommended minimum cup shape (>0.25), the diploid oysters generally had higher μCS.

In terms of fan shape, a significant two-way interaction between sample date and ploidy was observed. While all oysters sampled in September, regardless of ploidy, had similar μFS, by November diploid oysters show significantly higher μFS. In fact, the opposite was true for triploid oysters, which displayed a general decline, with the lowest μFS in the month of November. Overall, diploid oyster μFS ranged from 0.69 and 0.734, whereas, triploid oyster μFS ranged from 0.67 and 0.717 (see App. I, Table 1.1 – 1.6). While the ranges overlapped, and both diploid and triploid μFS were above the recommended minimum (>0.63), the diploid oysters generally had higher μFS.

Similar to μCS, the μFS data indicate an advantage in growing diploid oysters to achieve optimal fan shape. One explanation for this advantage could be the smaller overall size of diploids, allowing more room for growth across all stocking densities, which may enable higher μFS.

3.4.4 Condition Index

A significant three-way interaction was found among sample day, ploidy and flip on δCI. Throughout this study period, all oysters had excellent average condition index ratings. However, during the first two sample months (September and October) triploid mean δCI was significantly higher relative to diploid δCI (Tukey HSD: $p < 0.05$, see App. II, Table II.13). The triploid δCI started very high, and remained high, whereas diploids slowly increased in

δCI over time (section 3.3.4, see Figure 3.21). In November, triploid weekly flipped oysters mean δCI no longer significantly differed from weekly and biweekly diploid oysters mean δCI (Tukey HSD: p = 0.993, p = 0.368, see App.1, Table II.13). This growth in diploid δCI by the final sample could indicate diploid post-spawn recovery where increased efforts could have been directed towards tissue developed rather than gametes. Interestingly, this resembles the overall trend in diploid oyster ability to achieve the status of triploid oysters, albeit over a longer period of time.

Furthermore, a two-way interaction was found between ploidy and stocking density on δCI (ANOVA: p = 0.010, section 3.9, see Table 3.9, Figure 3.22). Triploids always had higher mean δCI compared to diploid oysters regardless of stocking density (Tukey HSD: p < 0.001, see App. II, Table 11.14). Within the diploid oysters, stocking density was not significant for mean δCIs, however, triploid oysters at 125 stocking density achieved significantly higher mean δCI values (125, δCI = 15.04) than those at the highest stocking density (175, δCI = 13.28) (Tukey HSD: p = 0.033, see App. II, Table II.14). Presumably, a reduction in stocking density may have helped with triploid oyster growth rates while achieving optimal condition index values, as these oysters are given more room to grow, with less competition for resources, as found previously in work by Rheault & Rice (1996). Additionally, a lower stocking density could provide more room for oysters to tumble within the bag, which could encourage deeper cup and wider fan. While the δCI values from this study indicate that triploids perhaps had higher δCI, it is important for farmers to consider the natural variables such as seasonal conditions, husbandry practices, and the physiological state of oysters (Newkirk, 1980; Allen & Downing, 1986).

3.4.5 Biofouling

A significant two-way interaction of flip and stocking density on µAFDW of biofouling organisms was observed (ANOVA: p = 0.002, section 3.3.5, see Figure 3.23). Across all treatments, there was a clear advantage for weekly flipped oysters in terms of reducing biofouling. Exposing oysters to air on a regular basis is a well-established biofouling mitigation method within the industry (Hooper, 2001) within this study, the major reduction of µAFDW in weekly flipped oysters clearly helps to reduce biomass accumulation on oysters regardless of ploidy, stocking density or sample date.

For all weekly flipped oysters, there was no effect of stocking density on µAFDW (Tukey HSD: $p = 1.000$, see App. II, Table II.15). However, for biweekly flipped oysters, 125 stocking density had significantly higher accumulation relative to 150 and 175 stocking density (Tukey HSD: $p < 0.05$, see App. II, Table II.15). One hypothesis for this could be that at lower stocking densities (i.e. 125), an increased water flow combined with more oyster shell surface area exposure allows for the significant increase in µAFDW. Conversely, while typically site specific and dependent on gear exposure and wave action, perhaps a lower stocking density would allow for increased tumbling and physical contact with other oysters, presumably reducing µAFDW by breaking off barnacles and other biofouling from individual shells. A site-specific and even seasonal investigation could help further explore the effects of stocking density on biofouling accumulation.

3.4.6 Percent Mortality

Finally, a significant effect of ploidy on mean percent mortality (µPM) indicates a higher mean µPM in diploid oysters (mean diploid= 6.9%, mean triploid = 2.8%, Figure 3.24, see App. II, Table II.16), with no other differences observed due to stocking density or flip regime. This higher mean µPM in diploid oysters is quite substantial and may be related to natural predation from oyster drills, crabs, or other predators (Flimlin & Beal, 1993) presumably because of smaller average size and strength. Another hypothesis is that reproductive diploid oysters may be in a state of stress, which could increase µPM related to environmental (predation and disease) and genetic (tolerance and gene mutation) factors, relative to the sterile triploid oyster. Past studies (Allen et al., 1993, Dégremont Garcia, Frank-Lawale & Allen, 2012) have indicated triploid oyster superior growth may provide increased protection against predation. Here it is also worth noting the importance of a lack of effects. There was no cost in terms of mortality to changing stocking densities or, interestingly, due to the flip regime.

3.5 Conclusion

The overall purpose of this study was to provide oyster farmers with tangible farm management techniques to enhance growth performance and quality control through the effect and interactions of ploidy, stocking density and flip regime for oysters grown in the FFCS.

Specifically, the following hypotheses were tested:

1. Concerning growth, shape and condition of oysters, it is hypothesized that:
   (a) Oyster ploidy will have a significant effect, such that on average triploid oysters will grow significantly larger relative to diploid oysters.
   (b) Stocking density will have a significant effect, such that 150 stocking density will be optimal.
   (c) The flip regime will not have a significant effect on growth, shape and condition of oysters.

2. Concerning biofouling it is hypothesized that:
   (a) Oyster ploidy will not have a significant effect on biofouling.
   (b) Stocking density will not have a significant effect on biofouling
   (c) The flip regime will have a significant effect on biofouling, where flipped oysters will have substantially less biofouling accumulation when compared to biweekly flipped oysters.

With regards to hypothesis 1 (a), Ploidy did have a significant effect on overall shell growth, such that triploid oysters were consistently larger than diploids over the course of the study. However, unexpectedly, triploids were often not as deeply cupped as diploid oysters, indicating the sheer growth advantage may hinder this essential shell characteristic (i.e. cup) associated with premium oysters. 1 (b), Stocking density did have a significant effect, yet the recommended 150 stocking density was not always optimal across all response variables. 1 (c), Unexpectedly, flip regime contributed to a number of significant interactions across the response variables of shell height, shell length and shell width as well as, dry tissue weight and condition index.

With regards to hypothesis 2 (a) As hypothesized, ploidy did not have a significant effect on biofouling. 2 (b) (c) Interestingly, an interactive effect of both stocking density and flip regime was found to significantly affect biofouling, such that weekly flipped oysters had significantly lower biofouling when compared with biweekly flipped oysters. Furthermore, where weekly flipped oysters did not significantly differ across stocking densities, biweekly flipped oysters stocked at 125 recorded significantly higher average biofouling, relative to 150 and 175.

It is clear from the results of this study, that oyster growth performance can be manipulated by each of the three factors tested. Depending on farming priorities and intended markets, farmers can employ the factors of ploidy, stocking density and flip regime to achieve the desired oyster.

4 Manuscript Two

Mitigation techniques for mud worm, Polydora websteri, infestation on farm-raised oysters

4.1 Introduction

The mud worm, *Polydora websteri*, is a marine polychaete that bores into the shells of many commercially important shellfish species (Lauckner, 1983; Handley & Bergquist, 1997). The earliest descriptions of *P. websteri* date back to the 1890s (Whitlegge 1890) and early 1900s (Morse, Rawson & Kraeuter, 2015), yet it was not until the early 1940s that *P. websteri* became labeled as an enemy of the *Crassostrea virginica* oyster industry (Lunz, 1940; Loosanoff & Engle, 1943).

Mud worms gain access to oysters as larvae and as they grow they begin to burrow into the shells creating 'mud blisters', which can negatively impact the perception, flavor and overall marketability of cultured oysters destined for the half-shell market (Littlewood et al., 1992 *as cited in* Morse et al. 2015; O'Sullivan, 1996 *as cited in* Davis, 2013; Handley and Bergquist, 1997). Past studies have investigated a variety of mud worm mitigation techniques that have shown various levels of success (Nel, Coetzee & Van Niekerk, 1996; Ghode & Kripa, 2001; Hooper, 2001; Dunphy, Wells & Jeffs, 2005; Davis, 2013), however, much of the Eastern oyster industry remains victim to mud worm infestation.

While several polychaete species of the genus *Polydora* are notorious 'shell borers' accused of damaging or even killing a variety of both wild and farmed shellfish (Bailey-Brock & Ringwood, 1982, Lauckner, 1983), this study focused on *P.* websteri infestation rates for off-bottom *C. virginica* oysters grown in the flippable floating cage System (FFCS), such has OysterGro™

4.1.1 Infestation, Damage & Response

The infestation of *P. websteri* begins with larval settlement on crevices found on the flat or cupped valves of the oyster shell (Zottoli & Carriker, 1974). Upon settlement, worms will build a mud tube on the surface of the shell and protrude inward, forming a U-shape burrow with both ends exposed to the outside environment (see Figure 4.1). Initially, the burrowing was believed to be merely an excavation process, however, Haigler (1969) provided evidence that shell penetration is achieved through a chemical secretion of "viscous

fluid" onto the burrow entrance that loosens the shell structure allowing for successful burrowing penetration (Haigler, 1969., Zottoli and Carriker, 1974).

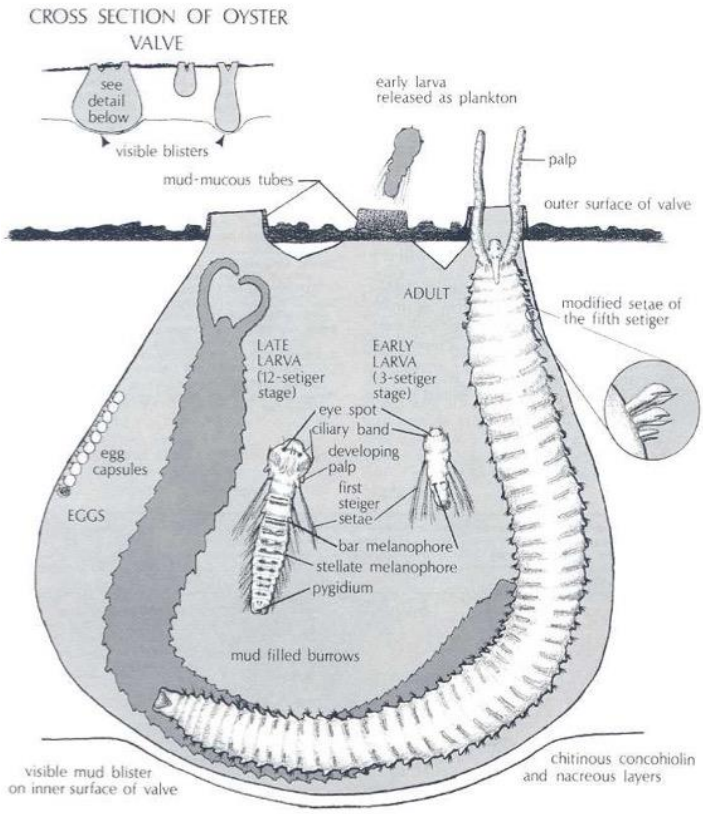

Figure 4.1 Schematic drawing of mud worm, P. websteri in the interior of a mud blister (from Bailey-Brock & Ringwood, 1982).

The seemingly defenseless oyster responds to the burrowing worm by depositing additional layers of the inner shell nacre to prevent further intrusion and possible contact with the internal tissue (Morse, et al. 2015). While oysters deposit nacre over the blisters, mud worms are simultaneously depositing detritus, mud, and fecal matter inside the burrow, creating a dark blister or "mud blister" (Bailey-Brock & Ringwood, 1982). Blisters cannot be seen until the oysters are opened (shucked), where *P. websteri* infestations are clearly visible on the inside of the shells, providing an unattractive alternative in comparison to the pearl white, blemish free oyster.

Figure 4.2 Photo of a shucked oyster with severe P. websteri infestation resulting in substantial mud blister damage to the interior of the shell.

Figure 4.3 Photo of an oyster that has no indication of P. websteri infestation, resulting in clean interior shells.

In addition to aesthetics, oysters that are heavily infested may be more prone to weakened shells, making shucking more challenging and often resulting in punctured blisters that can emit off-flavors and unpleasant scents (O'Sullivan, 1996; Morse et al. 2015). While oysters can often be successful in confining the worm, healing blisters and covering up any traces of infestation over time, the physical blister and additional shell nacre can disrupt feeding currents, reduce internal cavity volume and irritate the oyster throughout the physically demanding process (O'Sullivan, 1996, Dunphy et al. 2005).

4.1.2 Treatments

Past treatments for controlling infestations have mainly consisted of labour-intensive techniques that involve soaking oysters in solution, known as 'immersion techniques'. These solutions include and are not limited to; freshwater, saltwater (brine dips), hot water (70°C), formalin, and chlorine often in a combination of soaking procedures for a specified duration of time (MacKenzie & Shearer, 1959; Nel et al., 1996; Ghode & Kripa, 2001; Dunphy et al., 2005). Similar studies have looked into cold storage (3°C) techniques combined with an immersion solution, involve oysters being brought on land and kept in a cold storage facility (Brown, 2012). The various immersion techniques, cold storage methods, and a mixture of the two have had a varying degree of success with mud worm eradication (Ghode and Kripa, 2001; Dunphy et al., 2005, Brown, 2012). However, most of these treatments are typically

reactive methods that can be detrimental to the oysters and require a considerable about of time, money and labor.

Interestingly, Littlewood et al. (1992) suggested that regular aerial exposure of 40% or more can significantly reduce *P. websteri* infestation in *C. virginica* grown in rack and bag culture. Similar studies have investigated utilizing the tidal cycle for natural aerial exposure, which has proven successful for various regional and site-specific case studies (Bishop & Hooper, 2005, Brown, 2012). With advancements in floating and suspended grow out methods, farmers can now be in full control of the aerial exposure frequency and duration, allowing for a timely, cost-effective method for reducing or eliminating mud-blister worm infestations.

Fortunately, with an increase in innovation amongst oyster farmers and applied scientific research, and a growing demand and expectation in quality half-shell oysters, the mitigation techniques for mud worm infestations are becoming better understood. The competition and innovation amongst oyster farmers place special attention on mud worm mitigation techniques and strict quality control measures. While infestations are widespread across much of the cultured oyster industry in North America, the natural tidal cycle (Bishop & Hooper, 2005; Brown, 2012), does not always offer viable aerial exposure for mitigating mud worm infestation and other biofouling organisms. The increase in off-bottom oyster culture in the north-central GOM has created a need for effective, easily applicable and affordable approaches to mitigate mud worm infestation. This study investigates the pro-active implementation of regular desiccation (weekly or biweekly) for mitigating and controlling mud worms before the infestation becomes a problem.

4.1.3 Project Goal

The goal of this study was to determine a cost-effective and easily applicable method for addressing mud worm infestation on cultured oysters grown in the OysterGro™ floating cage system (FFCS). Implementing a low-cost, minimal labor and time sensitive farm management technique for the FFCS could help mitigate infestation rates and greatly reduce the damage caused by the mud worm, *P. websteri*. The results from this study could benefit current and future oyster farmers in the north-central GOM, as well as provide additional insight into the off-bottom industry as a whole.

The overarching question was to determine how the factors of ploidy, stocking density and flip regime impact mud worm, *P. websteri* infestation on oysters grown in the FFCS. Specifically, the following hypotheses were tested.

1. Concerning the abundance and dry weight of mud worm, *P. websteri* it is hypothesized that:
    a) Oyster ploidy will not have a significant effect on *P. websteri* abundance and dry worm weight.
    b) Stocking density will not have a significant effect on *P. websteri* abundance and dry worm weight.
    c) Flip regime will have a significant effect on *P. websteri* abundance and dry worm weight, such that weekly flipped oysters will have significantly lower *P. websteri* abundance and lower average dry worm weight compared to bi-weekly flipped oysters.

4.2 METHODS

4.2.1 Site Description

See 3.2.1

4.2.2 Gear Type: Floating Cage System

See 3.2.2

4.2.3 Experimental Design

This project took place over a three-month period, from August 25 to November 17, 2015. A fully factorial test of ploidy (2 levels), by stocking density (3 levels), by flip regime (2 levels) was developed for this study. The effects of these factors and their interactions were quantified through the response variables of worm abundance and dry worm weight. This experiment was conducted in conjunction with the study focusing on total percent mortality, shell growth, condition index and biofouling accumulation (see Manuscript 1).

The first factor for this study looked at a comparison between diploid and triploid oysters. Diploid and triploid oysters deployed for this project were entering the final grow out stage, making them ideal subjects for examining quality control techniques during the critical pre-harvest conditioning period. A total of 5400 (2700 diploid, 2700 triploid) oysters were needed for initial deployment.

The second factor assessed looked at a comparison of stocking densities for oysters grown in individual Vexar® bags deployed within the FFCS. Vexar® bags have a recommend stocking density of 150 oysters in the GOM (Davis et al., 2013). To assess the effect of increasing and decreasing the recommended stocking densities (~$\pm$17%), oysters were deployed at stocking densities of 125, 150, and 175, with three replicates per density.

The third factor assessed was the flip regime for oysters grown in the FFCS. Desiccation (accomplished with a flip of the cage) is a common quality control method used by farmers to minimize biofouling accumulation on culture gear and individual oysters. Based on work at Auburn University (Davis et al., 2013), it is recommended that the FFCS be desiccated weekly for a duration of ~24 hours (depending on air temperature). Here we tested whether there were differences between a flip regime of weekly and biweekly, holding the

desiccation duration constant. Three of the floating cages were randomly assigned to a weekly (24-hour duration) flip regime, and three were randomly assigned to a biweekly (24-hour duration) flip regime.

All oysters used for this study underwent the sample pre-deployment process of one wash and grading cycle through a QuickTube Sorter™ mechanical rotary style grader manufactured by the Chesapeake Bay Oyster Company, (see Manuscript One Figure 3.5). Oysters were processed through the aluminum 'market grading tube', which was manufactured with two hole sizes or 'grades', of 31.75 mm diameter and 44.45 mm diameter, with only the largest grade used for this study (Chesapeake Bay Oyster Company). The grader was also equipped with a spray wash bar connected to a freshwater supply, providing a steady stream of water for effective cleaning or 'tumblewash' of oysters processed through the grader. Once washed and graded (above 44.45 mm diameter), all oysters were then counted and divided by their respective stocking densities for deployment. Vexar® bags (18 diploids, 18 triploids) were identified with color-coded zip ties representing ploidy and stocking density. Individual bags were tagged accordingly and given a randomized placement within the FFCS to ensure unbiased experiment design.

On August 25, 2015, tagged bags were brought to AUORDF and deployed into six floating cages (large mesh - six pack model). The cages used throughout this project were previously deployed on the western most run of the AUORDF (See Manuscript One, Figure 3.6). The cages used throughout this project were previously deployed on the western most run of the AUORDF. From August 25 to November 17, weekly trips to the AUORDF were made every Monday and Tuesday morning in order to flip appropriate cages.

4.2.4 Sampling Protocol

Over the three-month study period, a destructive sampling process was used to record the response variables of worm count and dry worm weight. At three sample dates, September 22, October 20, November 17, oysters examined for mud worm infestation rates were taken in conjunction with samples utilized for the previously mentioned study (see Chapter 2). Transportation of samples from AUORDF to AUSL required 36 One Gallon Ziploc® freezer bags that kept samples separated and protected during transportation from field to lab. For record keeping and traceability, individual waterproof tags were created to accompany each sample bag on its way in from the field. All tags included a three-character code to display the

ploidy, flip regime and stocking density appointed to the individual sample (i.e. DB1 = Diploid, Biweekly, 125 density, and TW3 = Triploid, Weekly, 175 density). Of the 540 oysters sampled each month (15 oysters from 36 bags Vexar® bags) for the previously mentioned study (see Manuscript 1), 1 oyster from each Vexar® bag, totaling 36 oysters, were designated for mud worm analysis. Over the three-month period, the mud worm study examined 108 oysters for mud worm infestation.

4.2.5 Data Collection

Upon arriving at AUSL, oysters were separated into two groups, biweekly (18) and weekly (18). Eighteen 600ml Kimax beakers (Kimble, USA) and 36 Petri dishes were prepared. A seawater (instant ocean – 35ppt), phenol (500ppm) and dichlorobenzene (100ppm) solution was created to induce mud worms to leave their burrows, as used by (MacKenzie & Shearer 1959). Each beaker was filled with 250 ml of solution, where individual oysters were then submerged to soak for 12 ± 2 hours. As worms began escaping their burrows, forceps were used to extract the worms and put them into an assigned Petri dish.

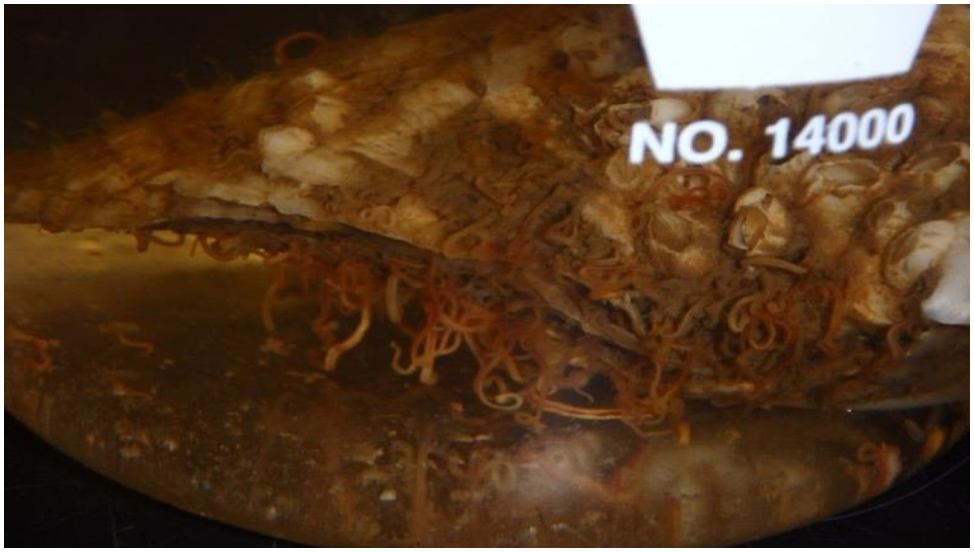

Figure 4.4 An oyster submerged for processing with substantial mud worm, P. websteri infestation.

To preserve worms prior to processing, 95% alcohol was added to each Petri dish. Waterproof tags were created to label Petri dishes, corresponding to the oyster's 3-character code used to identify treatments. After the first 18 oysters were processed, beakers were

washed and filled with new solution to prepare for the second sampling of oysters. Processing oysters occurred within 36 ± 4 hours after samples arrived at AUSL.

Once all 36 oysters were processed, worms were counted and assigned to a labeled 57 mm aluminum dish (VWR International). The aluminum dishes then went into a Fisher Scientific® ISOTEMP™ drying oven (Thermo Fisher Scientific Inc., Pittsburgh, PA) at 80°C for 48 ± 2 hours to collect a final dried worm weight.

4.2.6 Data Analysis

There was a total of twelve treatments (three ploidy x three stocking density x two flip regimes) with three replicates per treatment (36 bags total), sampled over three separate sampling periods. An ANOVA general linear model was employed to assess any interactions between the four factors (month, ploidy, stocking density, flip), for the response variable of worm count, and dry worm weight. For statistical purposes, the individual oyster worm counts and dry worm weights were used to calculate a numerical mean abundance value per treatment.

Systat® 13 (Systat Software Inc. Chicago, IL) and MiniTab® 17 (State College, PA) statistical software was used to analyze the data. All tests were performed with a significance level of $\alpha = 0.05$ where means were considered significantly different from one another if $p < 0.05$. Where significant interactions were found, a Tukey's post hoc pairwise comparison was performed to further explore results computed by the ANOVA.

4.3 Results

4.3.1 Worm Abundance

A significant two-way interaction between ploidy and flip was found to have an effect on worm abundance (µWA) (ANOVA: p = 0.020, Table 4.1, Figure 4.5), where there was no difference between diploid and triploids in the weekly flipped oysters, but biweekly flipped diploid oysters had significantly higher µWA when compared to biweekly flipped triploid oysters (Tukey HSD: $p < 0.05$, see App. III, Table III.1). Additionally, stocking density had no significant effect on µWA (ANOVA, p = 0.990, Table 4.1).

Table 4.1 ANOVA for mean worm abundance (µWA).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE_DATE	2	17191	8596	1.80	0.173
PLOIDY	**1**	**29074**	**29074**	**6.09**	**0.016**
FLIP	**1**	**248065**	**248065**	**51.97**	**0.000**
STOCKDENS	2	93	47	0.01	0.990
SAMPLE_DATE*PLOIDY	2	2772	1386	0.29	0.749
SAMPLE_DATE*FLIP	2	15407	7704	1.61	0.206
SAMPLE_DATE*STOCKDENS	4	6625	1656	0.35	0.845
PLOIDY*FLIP	**1**	**26822**	**26822**	**5.62**	**0.020**
PLOIDY*STOCKDENS	2	3747	1874	0.39	0.677
FLIP*STOCKDENS	2	211	106	0.02	0.978
SAMPLE_DATE*PLOIDY*FLIP	2	2376	1188	0.25	0.780
SAMPLE_DATE*PLOIDY*STOCKDENS	4	14767	3692	0.77	0.546
SAMPLE_DATE*FLIP*STOCKDENS	4	6082	1521	0.32	0.865
PLOIDY*FLIP*STOCKDENS	2	3381	1691	0.35	0.703
SAMPLE_DATE*PLOIDY*FLIP*STOCKDENS	4	14753	3688	0.77	0.547
Error	72	343651	4773		

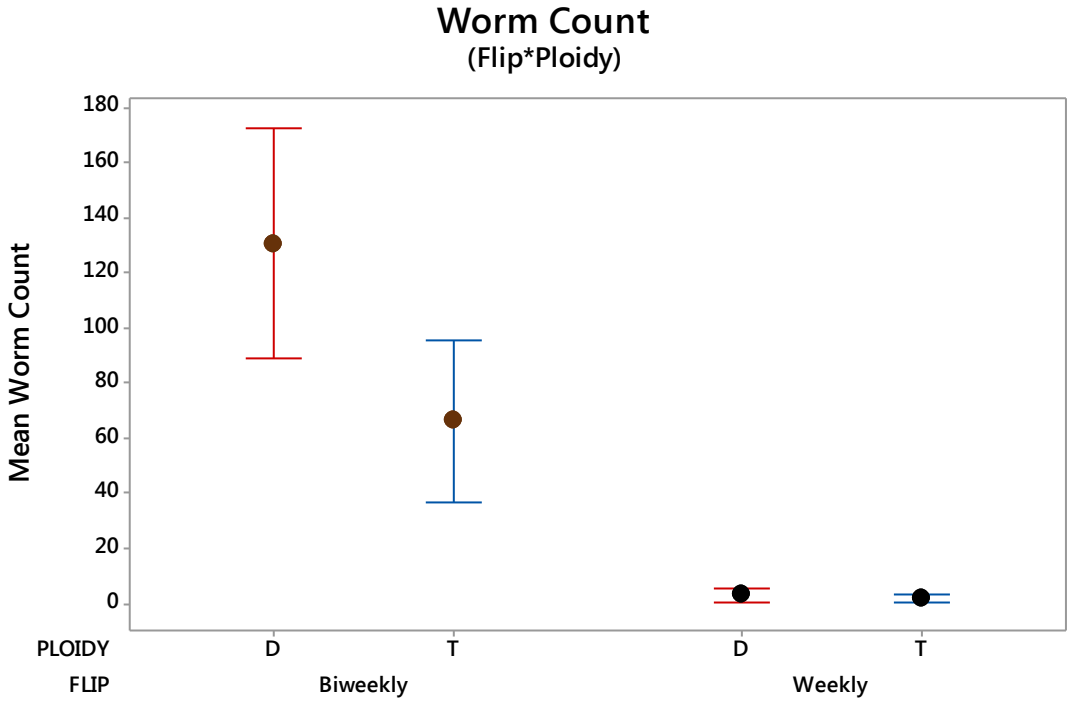

Figure 4.5 The significant effect of flip frequency and oyster ploidy on total worm abundance (µWA). Along the x-axis, D = diploid, T = triploid.

4.3.2 Dry Worm Weight

A significant two-way interaction between ploidy and flip was also found to have an effect on mean dry worm weight (µDWW) (ANOVA: $p = 0.014$, Table 4.2, Figure 4.6), where biweekly flipped oysters had significantly higher µDWW (g), than weekly flipped oysters (Tukey HSD: $p < 0.06$, see App. III, Table III.2). Interestingly, a significant two-way interaction between sampled date and flip was found to have an effect on µDWW (ANOVA: $p < 0.001$, Table 4.2, Figure 4.7), where bi-weekly flipped oysters had substantially higher µDWW, with a significant increase recorded over the three-month period (mean µDWW September = 0.02, October = 0.04, November = 0.12, see App. III, Table III.3).

Table 4.2 ANOVA for mean Dry Worm Weight (µDWW).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE_DATE	2	0.054122	0.027061	10.78	0.000
PLOIDY	1	0.017222	0.017222	6.86	0.011
FLIP	1	0.098283	0.098283	39.14	0.000
STOCKDENS	2	0.001368	0.000684	0.27	0.762
SAMPLE_DATE*PLOIDY	2	0.012636	0.006318	2.52	0.088
SAMPLE_DATE*FLIP	**2**	**0.053316**	**0.026658**	**10.62**	**0.000**
SAMPLE_DATE*STOCKDENS	4	0.010134	0.002533	1.01	0.409
PLOIDY*FLIP	**1**	**0.015895**	**0.015895**	**6.33**	**0.014**
PLOIDY*STOCKDENS	2	0.006704	0.003352	1.33	0.270
FLIP*STOCKDENS	2	0.001071	0.000535	0.21	0.809
SAMPLE_DATE*PLOIDY*FLIP	2	0.013235	0.006617	2.64	0.079
SAMPLE_DATE*PLOIDY*STOCKDENS	4	0.014468	0.003617	1.44	0.230
SAMPLE_DATE*FLIP*STOCKDENS	4	0.009021	0.002255	0.90	0.470
PLOIDY*FLIP*STOCKDENS	2	0.006296	0.003148	1.25	0.292
SAMPLE_DATE*PLOIDY*FLIP*STOCKDENS	4	0.015163	0.003791	1.51	0.208
Error	72	0.180811	0.002511		

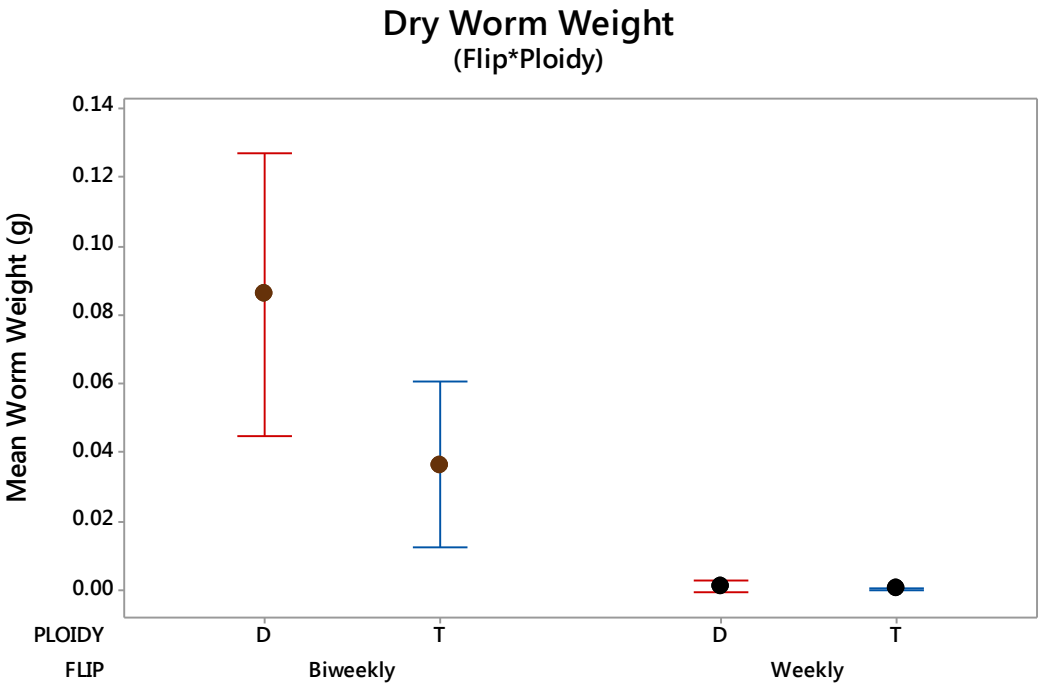

Figure 4.6 The significant effect of flip frequency and oyster ploidy on dry worm weight (µDWW).

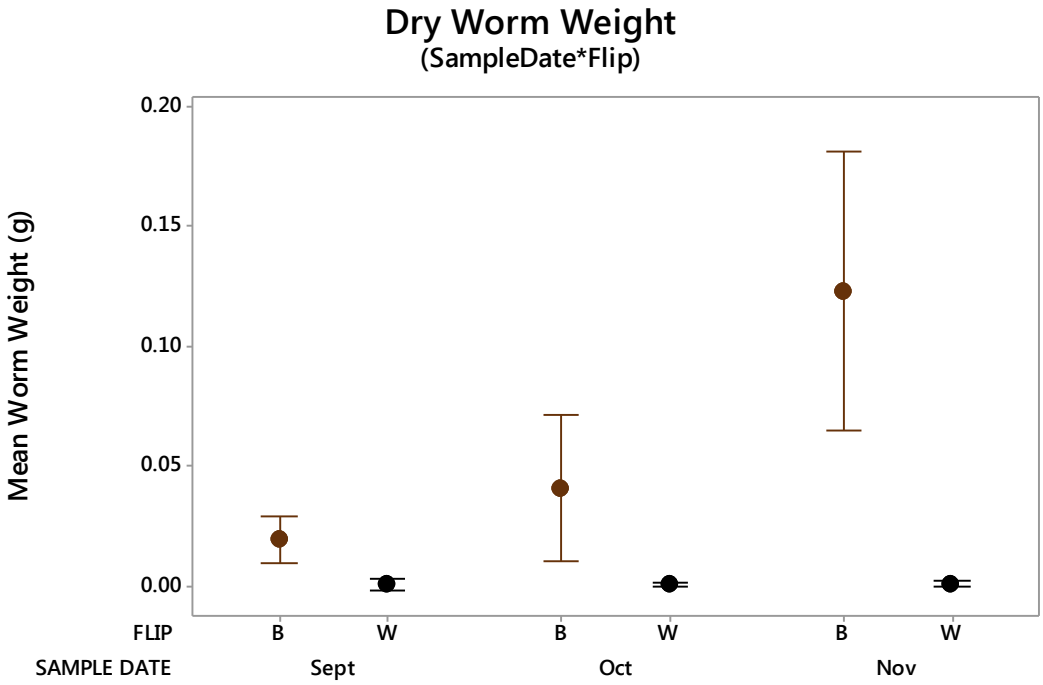

Figure 4.7 The significant effect of sample date and flip frequency on mean dry worm weight (µDWW).

4.4 Discussion

4.4.1 Worm Abundance

In this study, there was a clear benefit of flip frequency, where weekly flipping of oysters kept worm abundances low. Handley & Berquist (1997) found similar results associated with increased aerial exposure, where oysters showed significantly decreased numbers of recently settled worms, overall infestations and evidence of blisters on the shells interiors. While flipping gear on a weekly basis can cause slight growth reduction (see Manuscript 1), there are clear advantages for mud worm mitigation and quality control. With the incentive for a farmer to flip oysters on a biweekly schedule to shorten the growing season and increase production, heavy infestation of mud-blister worms can reduce the value of half-shell oysters, reducing demand, and can even lead to outright rejection of the product (Morse et al., 2015).

The importance of ploidy was apparent with biweekly flipped oysters, where diploid oysters had significantly higher µWA when compared with biweekly flipped triploids. Interestingly, the weekly flipped diploids did not significantly differ from weekly flipped

triploids, suggesting that regardless of ploidy, µWA can be kept under control through regular flipping. It is not clear what the mechanisms for the difference between diploids and triploids in the biweekly treatments, but one hypothesis for this is the faster growing triploid oyster could restrict the burrowing capabilities of newly settled mud worms. The second hypothesis for this could be that an increased biofouling accumulation on biweekly flipped oyster, combined with diploid slower average growth rate, may provide an increase in exterior shell crevices and a more malleable substrate for larvae to settle and begin the burrowing process, presumably with less resistance and higher success rate. Notably, while past studies (Loosanoff and Engle, 1943; Littlewood et al., 1992, Nel et al., 1996) suggest that stocking density and regular desiccation can help mitigate the abundance of mud worm infestations, no effect of stocking density was found in this study. This lack of response may be due to the relatively reserved changes in stocking densities (~$\pm$17% away from recommended) deployed for this project.

Furthermore, over the limited period of this study, no difference was found among sampling dates. An additional consideration of seasonal trends in *P. websteri* larvae abundance and settlement patterns may allow for a better understanding of infestation rates (Hopkins, 1958; Bailey-Brock & Ringwood, 1982). While seasonality and abundance of larvae population size have been documented in various other oyster-growing regions (Blake, 1969; Orth, 1971; Zajac, 1991; Nell, 2007) the settlement patterns of larvae in the GOM have not yet been documented. By understanding the life-cycle and seasonal trends in the GOM, farmers desiccation techniques can be synchronized to be employed when *P. websteri* are most volatile (larvae) or to mitigate initial settlement simply be flipping oysters during peak larvae abundance, thus minimizing labor and growth penalties.

4.4.2 Dry Worm Weight

The significant interaction of ploidy and flip on dry worm weight displayed similar trends found in the worm abundance, as was anticipated. However, the significant interaction of sample date and flip on µDWW could indicate that worms living within the oyster shells are growing over the three-month study period, allowing average biomass of individual worms to increase. Past studies have indicated that both an increase in mud worm size and residence time within the shell can lead to more severe internal mud blister damage (Lunz,1940; Nel et. al., 1996; Hooper, 2001), further stressing the need for farmers to proactively control mud worm infestation through regular weekly flip regimes

4.5 Conclusion

The overarching question was to determine how the factors of ploidy, stocking density and flip regime impact mud worm, *P. websteri* infestation on oysters grown in the FFCS. Specifically, the following hypotheses were tested.

1. Concerning the abundance and dry weight of mud worm, *P. websteri* it is hypothesized that:
    a) Oyster ploidy will not have a significant effect on *P. websteri* abundance and dry worm weight.
    b) Stocking density will not have a significant effect on *P. websteri* abundance and dry worm weight.
    c) Flip regime will have a significant effect on *P. websteri* abundance and dry worm weight, such that weekly flipped oysters will have significantly lower *P. websteri* abundance and lower average dry worm weight compared to bi-weekly flipped oysters.

With regards to hypothesis 1) (a) (c) interestingly, the significant interaction of ploidy and flip was found to have an effect on worm abundance and dry worm weight for bi-weekly flipped oysters, where diploids were found to have higher *P. websteri* abundance and dry worm weight relative to triploids. Furthermore, a significant effect of sample date and flip regime on mud worm weight, suggest an increase in average worm growth over the three-month period. (b) As hypothesized, stocking density did not have a significant effect on *P. websteri* abundance. (c) As hypothesized, flip was indeed found to have an effect on *P. websteri* abundance, such that weekly flipped oysters were found to have significantly lower *P. websteri* abundance, relative to biweekly flipped oysters.

In conclusion, weekly flipped oysters recorded significantly lower mud worm abundance when compared to biweekly flipped oysters, as anticipated. While diploid and triploid weekly flipped oysters did not differ in µWA, diploid biweekly flipped oysters had significantly higher µWA when compared to triploid biweekly oysters. Interestingly, the previous study (see Chapter 3) indicates many benefits associated with diploid oyster shell shape for the premium half-shell oyster industry, however, this study indicates, a higher mud worm abundance in diploid oysters, meaning without a regular weekly flip regime, diploid oysters may be more prone to mud worm infestations when compared to triploids.

5.0 Conclusion and Recommendations

5.1 Recommendations for Farm Management

The effect and interaction of sample date, ploidy, stocking density and flip regime had substantial impacts on oysters grown in the flippable floating cage system. Throughout all recorded response variables, there were a number of two, three and even a four-way interaction of factors, indicating that oysters grown in the FFCS are exposed to various influential factors that are often highly correlated. While all treatments used in this study produced high-quality oysters suitable for the premium half-shell industry, there were a number of overall trends identified amongst the tested factors that can serve as helpful recommendations for GOM farmers using the FFCS.

1) Triploids appear to offer faster growth and better condition index, but less cup shape, whereas, diploids appear to offer better overall shape, but slower growth and slightly higher mortality.
2) Weekly flipping did lead to a growth penalty, but there were clear benefits for reduced biofouling accumulation and average mud worm infestation.
3) Stocking density 150 looked to be a safe option with an improved cup over the three-month period and lowest average biofouling.

In conclusion, in order to remain competitive in the premium half-shell oyster industry, farmers in the north-central GOM should incorporate the above recommendation into their current farm management techniques. While the results do not provide farmers with a one size fits all best management guide or perfect ‘recipe’ for oyster grown in the FFCS, there were several beneficial and widely applicable farm level grow out techniques that GOM farmers could employ in the FFCS.

5.2 Recommendations for Research

Globally, there are numerous examples of shellfish industries that have farmed healthy, sustainable, and high-quality seafood for generations (Hargreaves, 2011). While the farming of bivalve filter feeding shellfish continues to be held up as a sustainable form of food production, it is important to better understand the demands that intensive aquaculture can have on the surrounding biophysical resources (Hall, Delaporte, Beveridge & O'Keefe, 2011). Furthermore, while this study took place on a pre-existing farm site, new farmers to the industry would need to consider various factors prior to developing a farm site and deploying culture gear (i.e. water depth, bottom characteristics, wave action, water quality, tidal flow and height, turbidity, predation, fouling, pollution, navigable waters, access, conflicts of uses, required permits, etc.) (Quayle & Newkirk, 1989). An oyster farmer that has developed a comprehensive understanding of the environment in which their oysters are grown will ultimately be in a better position for adopting farm management techniques that work in accordance with the surrounding environment.

For instance, the *C. virginica* off bottom culture industry is wide-spread across the Atlantic, Pacific and Gulf coastline of North America, and therefore, *C.virginica* is exposed to a variety of regional climate conditions and seasonal variability in weather patterns. While this study did not specifically test for the tracking and integration of local weather patterns into farming practices, the technique of scheduling flip regimes in accordance with regional climatic conditions is a developing field of study that could provide significant benefits for biofouling control and overall growth efficiency. As the results from this study indicate, the increase in flip frequency (weekly flip), may help mitigate negative impacts of biofouling and mud worm infestation, yet an associated decrease in shell growth may also occur. Developing a flip regime scheduled on ideal regional climate patterns, (i.e. peak sun exposure, low wind, low humidity), the duration of each flip could be reduced as oysters are more effectively desiccated, ultimately allowing more time for grow out.

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Appendix I

Chapters 3 & 4 Supporting Data

Table 1.1 - Summary of response variables (mean ± SEM) for flipping regime and stocking density of Diploid oysters sampled in September

Diploid	September					
	Bi			We		
	125	150	175	125	150	175
Shell Metrics						
Delta Shell Height (mm)	7.580 ± 1.770	8.753 ± 0.451	8.630 ± 1.180	9.411 ± 0.465	7.310 ± 2.110	8.082 ± 0.397
Delta Shell Length (mm)	4.680 ± 1.380	5.651 ± 0.296	6.431 ± 0.557	6.255 ± 0.099	5.065 ± 0.975	4.578 ± 0.338
Delta Shell Width (mm)	3.202 ± 0.727	3.597 ± 0.322	4.234 ± 0.333	3.935 ± 0.072	3.060 ± 1.040	2.934 ± 0.178
Delta Whole Wet Weight (g)	8.920 ± 2.700	9.300 ± 1.400	11.695 ± 0.480	11.905 ± 0.518	10.190 ± 2.760	10.040 ± 0.401
Delta Dry Shell Weight (g)	5.700 ± 1.850	5.428 ±0.968	7.440 ± 0.609	6.450 ± 1.040	6.390 ± 1.970	6.105 ± 0.470
Delta Dry Tissue Weight (g)	0.207 ± 0.070	0.289 ± 0.039	0.369 ±0.046	0.492 ± 0.044	0.430 ± 0.053	0.394 ± 0.025
Ratios						
Cup	0.357 ± 0.004	0.356 ± 0.004	0.357 ± 0.004	0.357 ± 0.003	0.354 ± 0.007	0.349 ± 0.002
Fan	0.703 ± 0.001	0.705 ± 0.003	0.720 ± 0.019	0.706 ± 0.008	0.713 ± 0.012	0.695 ± 0.002
Delta Condition Index	6.242 ± 0.339	7.426 ± 0.194	8.840 ± 1.370	9.380 ± 1.470	11.734 ± 0.967	10.084 ± 0.944
Biofouling						
Worm Count	117.30 ± 49.00	97.70 ± 49.80	74.70 ± 27.40	0.667 ± 0.667	0.667 ± 0.667	7.67 ± 7.67

Table 1.2 - Summary of response variables (mean ± SEM) for flipping regime and stocking density of Diploid oysters sampled in October

Diploid	October					
	Bi			We		
	125	150	175	125	150	175
Shell Metrics						
Delta Shell Height (mm)	17.199 ± 0.333	14.599 ± 0.476	19.497 ± 0.369	15.710 ± 1.550	18.790 ± 0.339	16.520 ± 1.100
Delta Shell Length (mm)	11.336 ± 0.674	10.111 ± 0.694	13.301 ± 0.419	11.430 ± 1.270	12.236± 0.323	11.390 ± 1.100
Delta Shell Width (mm)	7.434 ± 0.403	6.539 ± 0.291	7.703 ± 0.416	7.329 ± 0.287	6.971 ± 0.463	6.706 ± 0.193
Delta Whole Wet Weight (g)	24.300 ± 1.240	19.540 ± 1.110	27.067 ± 0.229	23.64 ± 2.030	24.780 ± 1.490	22.750 ± 1.640
Delta Dry Shell Weight (g)	15.190 ± 1.060	11.745 ± 0.660	17.041 ± 0.122	14.690 ± 1.160	15.720 ± 1.200	13.602 ± 0.998
Delta Dry Tissue Weight (g)	0.915 ± 0.056	0.804 ± 0.026	0.991 ± 0.015	0.879 ± 0.114	0.823 ± 0.114	0.83 ± 0.083
Ratios						
Cup	0.368 ± 0.005	0.370 ± 0.003	0.362 ± 0.005	0.376 ± 0.011	0.353 ± 0.008	0.361 ± 0.003
Fan	.703 ± 0.013	0.710 ± 0.011	0.707 ± 0.003	0.717 ± 0.014	0.698 ± 0.009	0.709 ± 0.014
Delta Condition Index	10.047 ± 0.548	10.362 ± 0.373	9.883 ± 0.092	9.792 ± 0.708	9.094 ± 0.468	9.042 ± 0.298
Biofouling						
Worm Count	75.00 ± 41.00	120.70 ± 68.50	180.00 ± 114.00	0.333 ± 0.333	2.00 ± 1.53	5.33 ± 5.33

Table - 1.3 Summary of response variables (mean ± SEM) for flipping regime and stocking density of Diploid oysters sampled in November

Diploid	November					
	Bi			We		
	125	150	175	125	150	175
Shell Metrics						
Delta Shell Height (mm)	25.091 ± 0.666	25.280 ± 1.890	24.790 ± 2.290	22.996 ± 0.851	24.291 ± 0.945	22.840 ± 1.410
Delta Shell Length (mm)	18.432 ± 0.431	18.923 ± 0.733	17.800 ± 1.310	17.412 ± 0.699	16.455 ± 0.314	17.550 ± 1.190
Delta Shell Width (mm)	10.517 ± 0.164	10.610 ± 0.370	9.086 ± 0.722	10.187 ± 0.389	10.138 ± 0.436	9.518 ± 0.462
Delta Whole Wet Weight (g)	37.600 ± 1.900	37.040 ± 1.890	35.090 ± 4.210	37.730 ± 0.979	38.320 ± 3.710	35.740 ± 4.450
Delta Dry Shell Weight (g)	22.880 ± 1.090	22.880 ± 1.190	21.240 ± 2.700	23.616 ± 0.436	24.300 ± 2.980	22.000 ± 2.180
Delta Dry Tissue Weight (g)	1.575 ± 0.109	1.397 ± 0.167	1.263 ± 0.146	1.593 ± 0.090	1.931 ± 0.115	1.784 ± 0.224
Ratios						
Cup	0.372 ± 0.003	0.373 ± 0.009	0.353 ± 0.002	0.378 ± 0.007	0.372 ± 0.003	0.370 ± 0.002
Fan	0.723 ± 0.007	0.729 ± 0.012	0.718 ± 0.004	0.730 ± 0.014	0.706 ± 0.004	0.734 ± 0.005
Delta Condition Index	10.675 ± 0.150	9.797 ± 0.728	9.106 ± 0.266	11.275 ± 0.322	13.785 ± 0.322	13.164 ± 0.694
Biofouling						
Worm Count	167.70 ± 34.90	175.00 ± 84.90	170.30 ± 85.90	7.00 ± 6.03	4.33 ± 2.60	1.00 ± 1.00
Ash Free Dry Weight (g)	0.105 ± 0.031	0.020 ± 0.006	0.034 ± 0.011	0.007 ± 0.002	0.006 ± 0.002	0.005 ± 0.001
Total Mortality	0.05 ± 0.02	0.08 ± 0.01	0.1 ± 0.02	0.06 ± 0.03	0.05 ± 0.01	0.05 ± 0.02

Table 1.4 Summary of response variables (mean ± SEM) for flipping regime and stocking density of triploid oysters sampled in September

Triploid	September					
	Bi			We		
	125	150	175	125	150	175
Shell Metrics						
Delta Shell Height (mm)	11.08 ± 1.420	11.711 ± 0.943	9.200 ± 1.950	8.830 ± 2.010	10.930 ± 2.370	9.200 ± 2.820
Delta Shell Length (mm)	6.775 ± 0.132	7.963 ± 0.727	7.340 ± 0.631	6.330 ± 1.590	7.356 ± 0.734	6.763 ± 0.959
Delta Shell Width (mm)	3.163 ± 0.135	2.904 ± 0.381	2.785 ± 0.489	2.713 ± 0.768	3.783 ± 0.176	3.901 ± 0.592
Delta Whole Wet Weight (g)	18.860 ± 1.900	19.240 ± 1.520	16.140 ± 3.350	18.990 ±4.290	24.220 ± 1.690	21.230 ± 4.150
Delta Dry Shell Weight (g)	12.530 ± 1.140	13.050 ± 1.220	11.250 ± 1.890	13.260 ± 2.750	16.508 ± 0.816	14.540 ± 2.700
Delta Dry Tissue Weight (g)	0.970 ± 0.100	0.809 ± 0.081	0.717 ± 0.155	0.920 ± 0.136	1.0369 ± 0.065	0.959 ± 0.150
Ratios						
Cup	0.337 ± 0.006	0.331 ± 0.006	0.341 ± 0.003	0.340 ± 0.005	0.346 ± 0.013	0.356 ± 0.009
Fan	0.688 ± 0.012	0.699 ± 0.012	0.715 ± 0.011	0.701 ± 0.005	0.700 ± 0.018	0.706 ± 0.014
Condition Index	15.392 ± 0.476	13.050 ± 1.140	15.650 ± 2.370	17.920 ± 3.410	13.599 ± 0.663	14.780 ± 1.030
Biofouling						
Worm Count	3.67 ± 2.67	83.30 ± 43.40	18.67 ± 4.84	0	0.333 ± 0.333	0.667 ± 0.667

Table. 1.5 Summary of response variables (mean ± SEM) for flipping regime and stocking density of triploid oysters sampled in October

Triploid	October					
	Bi			We		
	125	150	175	125	150	175
Shell Metrics						
Delta Shell Height (mm)	18.550 ± 1.590	19.155 ± 0.484	15.830 ± 1.490	14.682 ± 0.412	16.95 ± 1.170	18.543 ± 0.816
Delta Shell Length (mm)	11.840 ± 1.450	12.347 ± 0.346	12.312 ± 0.673	10.960 ± 0.368	11.692 ± 0.490	11.326 ± 0.795
Delta Shell Width (mm)	6.659 ± 0.465	6.441 ± 0.450	6.495 ± 0.146	5.551 ± 0.145	5.866 ± 0.446	6.019 ± 0.185
Delta Whole Wet Weight (g)	38.170 ± 1.600	38.452 ± 0.980	36.970 ± 2.850	33.730 ± 1.020	39.068 ± 0.871	39.790 ± 2.340
Delta Dry Shell Weight (g)	25.190 ± 1.400	25.589 ± 0.761	24.370 ± 1.720	23.33 ± 1.120	26.459 ± 0.578	27.660 ± 1.640
Delta Dry Tissue Weight (g)	1.681 ± 0.139	1.659 ± 0.044	1.503 ± 0.117	1.523 ± 0.055	1.670 ± 0.055	1.709 ± 0.094
Ratios						
Cup	0.350 ± 0.013	0.345 ± 0.004	0.360 ± 0.006	0.353 ± 0.003	0.348 ± 0.010	0.343 ± 0.004
Fan	0.687 ± 0.030	0.689 ± 0.007	0.717 ± 0.004	0.708 ± 0.007	0.698 ± 0.008	0.681 ± 0.004
Condition Index	12.926 ± 0.854	12.893 ± 0.121	11.946 ± 0.144	14.827 ± 0.942	13.257 ± 0.464	14.119 ± 0.343
Biofouling						
Worm Count	117.30 ± 94.80	77.00 ± 39.20	55.00 ± 38.00	2.67 ± 2.67	1.00 ± 1.00	5.33 ± 2.91

Table 1.6 - Summary of response variables (mean ± SEM) for flipping regime, stocking density of triploid oysters sampled in November

Triploid	November					
	Bi			We		
	125	150	175	125	150	175
Shell Metrics						
Delta Shell Height (mm)	29.858 ± 0.950	30.170 ± 1.070	32.940 ± 1.430	27.791 ± 0.782	26.070 ± 3.910	28.903 ± 0.510
Delta Shell Length (mm)	19.501 ± 0.200	19.549 ± 0.305	20.777 ± 0.718	18.736 ± 0.694	17.370 ± 1.320	18.236 ± 0.625
Delta Shell Width (mm)	9.776 ± 0.408	9.638 ± 0.607	10.404 ± 0.404	10.165 ± 0.165	9.977 ± 0.701	9.409 ± 0.206
Delta Whole Wet Weight (g)	66.42 ± 2.840	64.270 ± 1.770	73.600 ± 2.660	70.580 ± 2.340	64.010 ± 6.380	67.200 ± 1.560
Delta Dry Shell Weight (g)	44.050 ± 2.310	41.620 ± 1.710	48.020 ± 2.140	47.000 ± 2.430	43.250 ± 4.550	45.100 ± 1.070
Delta Dry Tissue Weight (g)	3.807 ± 0.136	3.836 ± 0.124	3.119 ± 0.417	2.860 ± 0.048	2.519 ± 0.155	2.448 ± 0.025
Ratios						
Cup	0.340 ± 0.008	0.338 ± 0.010	0.337 ± 0.008	0.353 ± 0.005	0.358 ± 0.009	0.340 ± 0.001
Fan	0.686 ± 0.007	0.684 ± 0.012	0.677 ± 0.004	0.693 ± 0.008	0.692 ± 0.018	0.680 ± 0.005
Condition Index	17.018 ± 0.317	16.933 ± 0.523	12.130 ± 1.260	12.127 ± 0.131	12.350 ± 1.360	11.082 ± 0.180
Biofouling						
Worm Count	129.30 ± 38.20	36.00 ± 18.00	76.00 ± 23.90	3.67 ± 2.73	1.67 ± 0.88	2.00 ± 2.00
Ash Free Dry Weight (g)	0.068 ± 0.021	0.034 ± 0.010	0.057 ± 0.018	0.003 ± N/A	0.006 ± 0.006	0.021 ± N/A
Total Mortality	0.02 ± 0.01	0.01 ± 0.002	0.04 ± 0.02	0.03 ± 0.01	0.03 ± 0.005	0.03 ± 0.005

APPENDIX II

Chapter 3 Supporting Data

Table II.1

Tukey Pairwise Comparisons for Delta Shell Height
Interaction of Sample Date*Ploidy
Grouping Information Using the Tukey Method and 95% Confidence

SAMPLE DATE*PLOIDY	N	Mean	Grouping
42324 T	18	29.2896	A
42324 D	18	24.2139	B
42296 T	18	17.2850	C
42296 D	18	17.0523	C
42268 T	18	10.1604	D
42268 D	18	8.2937	D

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of SAMPLE DATE*PLOIDY Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(42268 T) - (42268 D)	**1.867**	**0.852**	**(-0.628, 4.361)**	**2.19**	**0.255**
(42296 D) - (42268 D)	8.759	0.852	(6.264, 11.253)	10.28	0.000
(42296 T) - (42268 D)	8.991	0.852	(6.497, 11.486)	10.55	0.000
(42324 D) - (42268 D)	15.920	0.852	(13.426, 18.415)	18.68	0.000
(42324 T) - (42268 D)	20.996	0.852	(18.501, 23.491)	24.64	0.000
(42296 D) - (42268 T)	6.892	0.852	(4.397, 9.387)	8.09	0.000
(42296 T) - (42268 T)	7.125	0.852	(4.630, 9.619)	8.36	0.000
(42324 D) - (42268 T)	14.053	0.852	(11.559, 16.548)	16.49	0.000
(42324 T) - (42268 T)	19.129	0.852	(16.635, 21.624)	22.45	0.000
(42296 T) - (42296 D)	**0.233**	**0.852**	**(-2.262, 2.727)**	**0.27**	**1.000**
(42324 D) - (42296 D)	7.162	0.852	(4.667, 9.656)	8.40	0.000
(42324 T) - (42296 D)	12.237	0.852	(9.743, 14.732)	14.36	0.000
(42324 D) - (42296 T)	6.929	0.852	(4.434, 9.424)	8.13	0.000
(42324 T) - (42296 T)	12.005	0.852	(9.510, 14.499)	14.09	0.000
(42324 T) - (42324 D)	5.076	0.852	(2.581, 7.570)	5.96	0.000

Table II.2

Tukey Pairwise Comparisons for Delta Shell Height
Single Factor of Flip
Grouping Information Using the Tukey Method and 95% Confidence

FLIP	N	Mean	Grouping
B	54	18.3288	A
W	54	17.1028	B

Means that do not share a letter are significantly different.
Tukey Simultaneous Tests for Differences of Means

Difference of FLIP Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
W - B	-1.226	0.492	(-2.207, -0.245)	-2.49	0.015

Table II.3

Tukey Pairwise Comparisons for Delta Shell Length
Single Factor of Sample Date
Grouping Information Using the Tukey Method and 95% Confidence

SAMPLE DATE	N	Mean	Grouping
42324	36	18.3949	A
42296	36	11.6904	B
42268	36	6.2663	C

Means that do not share a letter are significantly different.
Tukey Simultaneous Tests for Differences of Means

Difference of SAMPLE DATE Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
42296 - 42268	5.424	0.331	(4.632, 6.216)	16.37	0.000
42324 - 42268	12.129	0.331	(11.337, 12.921)	36.60	0.000
42324 - 42296	6.704	0.331	(5.912, 7.497)	20.23	0.000

Table II.4

Tukey Pairwise Comparisons for Delta Shell Length
Single Factor of Ploidy
Grouping Information Using the Tukey Method and 95% Confidence

PLOIDY	N	Mean	Grouping
T	54	12.6212	A
D	54	11.6132	B

Means that do not share a letter are significantly different.
Tukey Simultaneous Tests for Differences of Means

Difference of PLOIDY Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
T - D	1.008	0.271	(0.468, 1.547)	3.72	0.000

Table II.5

Tukey Pairwise Comparisons for Delta Shell Length
Single Factor of Flip
Grouping Information Using the Tukey Method and 95% Confidence

FLIP	N	Mean	Grouping
B	54	12.5042	A
W	54	11.7302	B

Means that do not share a letter are significantly different.
Tukey Simultaneous Tests for Differences of Means

Difference of FLIP Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
W - B	-0.774	0.271	(-1.313, -0.235)	-2.86	0.006

Table II.6

Tukey Pairwise Comparisons for Delta Shell Width
Interaction of Sample Date*Ploidy*Flip*Stocking Density

Grouping Information Using the Tukey Method and 95% Confidence (Four Way Interaction)

SAMPLE DATE*PLOIDY*FLIP*STOCKING DENSITY	N	Mean	Grouping
42324 D B 2	3	10.6096	A
42324 D B 1	3	10.5170	A
42324 T B 3	3	10.4044	A
42324 D W 1	3	10.1873	A B
42324 T W 1	3	10.1648	A B
42324 D W 2	3	10.1383	A B
42324 T W 2	3	9.9773	A B
42324 T B 1	3	9.7759	A B C
42324 T B 2	3	9.6378	A B C
42324 D W 3	3	9.5178	A B C
42324 T W 3	3	9.4087	A B C D
42324 D B 3	3	9.0860	A B C D E
42296 D B 3	3	7.7033	B C D E F
42296 D B 1	3	7.4341	C D E F
42296 D W 1	3	7.3294	C D E F
42296 D W 2	3	6.9710	D E F
42296 D W 3	3	6.7057	E F G
42296 T B 1	3	6.6594	E F G
42296 D B 2	3	6.5393	F G
42296 T B 3	3	6.4951	F G
42296 T B 2	3	6.4408	F G H
42296 T W 3	3	6.0189	F G H I
42296 T W 2	3	5.8661	F G H I
42296 T W 1	3	5.5510	F G H I J
42268 D B 3	3	4.2344	G H I J K
42268 D W 1	3	3.9345	H I J K
42268 T W 3	3	3.9009	I J K
42268 T W 2	3	3.7827	I J K
42268 D B 2	3	3.5972	I J K
42268 D B 1	3	3.2025	J K
42268 T B 1	3	3.1632	J K
42268 D W 2	3	3.0591	J K
42268 D W 3	3	2.9340	K
42268 T B 2	3	2.9041	K
42268 T B 3	3	2.7849	K
42268 T W 1	3	2.7135	K

Means that do not share a letter are significantly different.

Table II.7

Tukey Pairwise Comparisons for Delta Whole Wet Weight
Interaction of Sample Date*Ploidy

Grouping Information Using the Tukey Method and 95% Confidence

SAMPLE DATE*PLOIDY	N	Mean	Grouping
42324 T	18	67.6806	A
42296 T	18	37.6971	B
42324 D	18	36.9215	B
42296 D	18	23.6787	C
42268 T	18	19.7805	C
42268 D	18	10.3414	D

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of SAMPLE DATE*PLOIDY Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(42268 T) - (42268 D)	9.44	1.46	(5.17, 13.71)	6.47	0.000
(42296 D) - (42268 D)	13.34	1.46	(9.06, 17.61)	9.14	0.000
(42296 T) - (42268 D)	27.36	1.46	(23.08, 31.63)	18.74	0.000
(42324 D) - (42268 D)	26.58	1.46	(22.31, 30.85)	18.21	0.000
(42324 T) - (42268 D)	57.34	1.46	(53.07, 61.61)	39.28	0.000
(42296 D) - (42268 T)	**3.90**	**1.46**	**(-0.38, 8.17)**	**2.67**	**0.094**
(42296 T) - (42268 T)	17.92	1.46	(13.64, 22.19)	12.27	0.000
(42324 D) - (42268 T)	17.14	1.46	(12.87, 21.41)	11.74	0.000
(42324 T) - (42268 T)	47.90	1.46	(43.63, 52.17)	32.81	0.000
(42296 T) - (42296 D)	14.02	1.46	(9.75, 18.29)	9.60	0.000
(42324 D) - (42296 D)	13.24	1.46	(8.97, 17.52)	9.07	0.000
(42324 T) - (42296 D)	44.00	1.46	(39.73, 48.28)	30.14	0.000
(42324 D) - (42296 T)	**-0.78**	**1.46**	**(-5.05, 3.50)**	**-0.53**	**0.995**
(42324 T) - (42296 T)	29.98	1.46	(25.71, 34.26)	20.54	0.000
(42324 T) - (42324 D)	30.76	1.46	(26.49, 35.03)	21.07	0.000

Table II.8

Tukey Pairwise Comparisons for Delta Dry Shell Weight
Interaction of Sample Date*Ploidy

Grouping Information Using the Tukey Method and 95% Confidence

SAMPLE DATE*PLOIDY	N	Mean	Grouping
42324 T	18	44.8385	A
42296 T	18	25.4327	B
42324 D	18	22.8192	B
42296 D	18	14.6646	C
42268 T	18	13.5238	C
42268 D	18	6.2524	D

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of SAMPLE DATE*PLOIDY Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(42268 T) - (42268 D)	7.27	1.01	(4.30, 10.24)	7.17	0.000
(42296 D) - (42268 D)	8.41	1.01	(5.44, 11.38)	8.29	0.000
(42296 T) - (42268 D)	19.18	1.01	(16.21, 22.15)	18.91	0.000
(42324 D) - (42268 D)	16.57	1.01	(13.60, 19.54)	16.33	0.000
(42324 T) - (42268 D)	38.59	1.01	(35.62, 41.56)	38.04	0.000
(42296 D) - (42268 T)	**1.14**	**1.01**	**(-1.83, 4.11)**	**1.12**	**0.870**
(42296 T) - (42268 T)	11.91	1.01	(8.94, 14.88)	11.74	0.000
(42324 D) - (42268 T)	9.30	1.01	(6.33, 12.27)	9.16	0.000
(42324 T) - (42268 T)	31.31	1.01	(28.34, 34.28)	30.87	0.000
(42296 T) - (42296 D)	10.77	1.01	(7.80, 13.74)	10.61	0.000
(42324 D) - (42296 D)	8.15	1.01	(5.18, 11.12)	8.04	0.000
(42324 T) - (42296 D)	30.17	1.01	(27.20, 33.14)	29.74	0.000
(42324 D) - (42296 T)	**-2.61**	**1.01**	**(-5.58, 0.36)**	**-2.58**	**0.117**
(42324 T) - (42296 T)	19.41	1.01	(16.44, 22.38)	19.13	0.000
(42324 T) - (42324 D)	22.02	1.01	(19.05, 24.99)	21.71	0.000

Table II.9

Tukey Pairwise Comparisons for Delta Dry Tissue Weight
Interaction of Sample Date*Ploidy*Flip
Grouping Information Using the Tukey Method and 95% Confidence

SAMPLE DATE*PLOIDY*FLIP	N	Mean	Grouping				
42324 T B	9	3.58752	A				
42324 T W	9	2.60916		B			
42324 D W	9	1.76932			C		
42296 T W	9	1.63370			C	D	
42296 T B	9	1.61428			C	D	
42324 D B	9	1.41175				D	
42268 T W	9	0.97218					E
42296 D B	9	0.90343					E
42296 D W	9	0.84391					E
42268 T B	9	0.83204					E
42268 D W	9	0.43865						F
42268 D B	9	0.28827						F

Means that do not share a letter are significantly different.
Tukey Simultaneous Tests for Differences of Means

Difference of SAMPLE DATE*PLOIDY*FLIP Levels	Difference of Means	SE of Difference	Simultaneous 95% CI		T-Value	Adjusted P-Value
(42268 D W) - (42268 D B)	**0.150**	**0.100**	**(-0.188,**	**0.489)**	**1.50**	**0.935**
(42268 T B) - (42268 D B)	0.544	0.100	(0.205,	0.882)	5.43	0.000
(42268 T W) - (42268 D B)	0.684	0.100	(0.345,	1.022)	6.83	0.000
(42296 D B) - (42268 D B)	0.615	0.100	(0.277,	0.954)	6.14	0.000
(42296 D W) - (42268 D B)	0.556	0.100	(0.217,	0.894)	5.55	0.000
(42296 T B) - (42268 D B)	1.326	0.100	(0.988,	1.664)	13.24	0.000
(42296 T W) - (42268 D B)	1.345	0.100	(1.007,	1.684)	13.44	0.000
(42324 D B) - (42268 D B)	1.123	0.100	(0.785,	1.462)	11.22	0.000
(42324 D W) - (42268 D B)	1.481	0.100	(1.143,	1.820)	14.79	0.000
(42324 T B) - (42268 D B)	3.299	0.100	(2.961,	3.638)	32.95	0.000
(42324 T W) - (42268 D B)	2.321	0.100	(1.982,	2.659)	23.18	0.000
(42268 T B) - (42268 D W)	0.393	0.100	(0.055,	0.732)	3.93	0.010
(42268 T W) - (42268 D W)	0.534	0.100	(0.195,	0.872)	5.33	0.000
(42296 D B) - (42268 D W)	0.465	0.100	(0.126,	0.803)	4.64	0.001
(42296 D W) - (42268 D W)	0.405	0.100	(0.067,	0.744)	4.05	0.007
(42296 T B) - (42268 D W)	1.176	0.100	(0.837,	1.514)	11.74	0.000
(42296 T W) - (42268 D W)	1.195	0.100	(0.857,	1.534)	11.93	0.000
(42324 D B) - (42268 D W)	0.973	0.100	(0.635,	1.312)	9.72	0.000
(42324 D W) - (42268 D W)	1.331	0.100	(0.992,	1.669)	13.29	0.000
(42324 T B) - (42268 D W)	3.149	0.100	(2.810,	3.487)	31.45	0.000
(42324 T W) - (42268 D W)	2.171	0.100	(1.832,	2.509)	21.68	0.000
(42268 T W) - (42268 T B)	**0.140**	**0.100**	**(-0.198,**	**0.479)**	**1.40**	**0.960**
(42296 D B) - (42268 T B)	**0.071**	**0.100**	**(-0.267,**	**0.410)**	**0.71**	**1.000**
(42296 D W) - (42268 T B)	**0.012**	**0.100**	**(-0.327,**	**0.350)**	**0.12**	**1.000**
(42296 T B) - (42268 T B)	0.782	0.100	(0.444,	1.121)	7.81	0.000
(42296 T W) - (42268 T B)	0.802	0.100	(0.463,	1.140)	8.01	0.000
(42324 D B) - (42268 T B)	0.580	0.100	(0.241,	0.918)	5.79	0.000
(42324 D W) - (42268 T B)	0.937	0.100	(0.599,	1.276)	9.36	0.000
(42324 T B) - (42268 T B)	2.755	0.100	(2.417,	3.094)	27.52	0.000
(42324 T W) - (42268 T B)	1.777	0.100	(1.439,	2.116)	17.75	0.000
(42296 D B) - (42268 T W)	**-0.069**	**0.100**	**(-0.407,**	**0.270)**	**-0.69**	**1.000**
(42296 D W) - (42268 T W)	**-0.128**	**0.100**	**(-0.467,**	**0.210)**	**-1.28**	**0.979**
(42296 T B) - (42268 T W)	0.642	0.100	(0.304,	0.981)	6.41	0.000
(42296 T W) - (42268 T W)	0.662	0.100	(0.323,	1.000)	6.61	0.000
(42324 D B) - (42268 T W)	0.440	0.100	(0.101,	0.778)	4.39	0.002
(42324 D W) - (42268 T W)	0.797	0.100	(0.459,	1.136)	7.96	0.000
(42324 T B) - (42268 T W)	2.615	0.100	(2.277,	2.954)	26.12	0.000
(42324 T W) - (42268 T W)	1.637	0.100	(1.299,	1.975)	16.35	0.000
(42296 D W) - (42296 D B)	**-0.060**	**0.100**	**(-0.398,**	**0.279)**	**-0.59**	**1.000**
(42296 T B) - (42296 D B)	0.711	0.100	(0.372,	1.049)	7.10	0.000
(42296 T W) - (42296 D B)	0.730	0.100	(0.392,	1.069)	7.29	0.000
(42324 D B) - (42296 D B)	0.508	0.100	(0.170,	0.847)	5.08	0.000
(42324 D W) - (42296 D B)	0.866	0.100	(0.527,	1.204)	8.65	0.000
(42324 T B) - (42296 D B)	2.684	0.100	(2.346,	3.023)	26.80	0.000
(42324 T W) - (42296 D B)	1.706	0.100	(1.367,	2.044)	17.03	0.000
(42296 T B) - (42296 D W)	0.770	0.100	(0.432,	1.109)	7.69	0.000
(42296 T W) - (42296 D W)	0.790	0.100	(0.451,	1.128)	7.89	0.000
(42324 D B) - (42296 D W)	0.568	0.100	(0.229,	0.906)	5.67	0.000
(42324 D W) - (42296 D W)	0.925	0.100	(0.587,	1.264)	9.24	0.000
(42324 T B) - (42296 D W)	2.744	0.100	(2.405,	3.082)	27.40	0.000
(42324 T W) - (42296 D W)	1.765	0.100	(1.427,	2.104)	17.63	0.000
(42296 T W) - (42296 T B)	**0.019**	**0.100**	**(-0.319,**	**0.358)**	**0.19**	**1.000**
(42324 D B) - (42296 T B)	**-0.203**	**0.100**	**(-0.541,**	**0.136)**	**-2.02**	**0.677**
(42324 D W) - (42296 T B)	**0.155**	**0.100**	**(-0.183,**	**0.494)**	**1.55**	**0.921**
(42324 T B) - (42296 T B)	1.973	0.100	(1.635,	2.312)	19.71	0.000
(42324 T W) - (42296 T B)	0.995	0.100	(0.656,	1.333)	9.94	0.000
(42324 D B) - (42296 T W)	**-0.222**	**0.100**	**(-0.560,**	**0.117)**	**-2.22**	**0.544**
(42324 D W) - (42296 T W)	**0.136**	**0.100**	**(-0.203,**	**0.474)**	**1.35**	**0.968**
(42324 T B) - (42296 T W)	1.954	0.100	(1.615,	2.292)	19.51	0.000
(42324 T W) - (42296 T W)	0.975	0.100	(0.637,	1.314)	9.74	0.000
(42324 D W) - (42324 D B)	**0.358**	**0.100**	**(0.019,**	**0.696)**	**3.57**	**0.029**
(42324 T B) - (42324 D B)	2.176	0.100	(1.837,	2.514)	21.73	0.000
(42324 T W) - (42324 D B)	1.197	0.100	(0.859,	1.536)	11.96	0.000
(42324 T B) - (42324 D W)	1.818	0.100	(1.480,	2.157)	18.16	0.000
(42324 T W) - (42324 D W)	0.840	0.100	(0.501,	1.178)	8.39	0.000
(42324 T W) - (42324 T B)	-0.978	0.100	(-1.317,	-0.640)	-9.77	0.000

Individual confidence level = 99.88%

Table II.10

Tukey Pairwise Comparisons for Mean Cup Shape
Interaction of Sample Date*Stocking Density

Grouping Information Using the Tukey Method and 95% Confidence

SAMPLE DATE*STOCKING DENSITY	N	Mean	Grouping		
42296 1	12	0.361658	A		
42324 1	12	0.360753	A		B
42324 2	12	0.360086	A		B
42296 3	12	0.356384	A		B
42296 2	12	0.353875	A		B
42268 3	12	0.353416	A		B
42324 3	12	0.349831	A		B
42268 1	12	0.347779	A		B
42268 2	12	0.346714			B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of SAMPLE DATE*STOCKING DENSITY Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(42268 2) - (42268 1)	-0.00106	0.00461	(-0.01578, 0.01365)	-0.23	1.000
(42268 3) - (42268 1)	0.00564	0.00461	(-0.00908, 0.02036)	1.22	0.948
(42296 1) - (42268 1)	0.01388	0.00461	(-0.00084, 0.02860)	3.01	0.080
(42296 2) - (42268 1)	0.00610	0.00461	(-0.00862, 0.02082)	1.32	0.921
(42296 3) - (42268 1)	0.00861	0.00461	(-0.00611, 0.02332)	1.87	0.637
(42324 1) - (42268 1)	0.01297	0.00461	(-0.00175, 0.02769)	2.82	0.128
(42324 2) - (42268 1)	0.01231	0.00461	(-0.00241, 0.02703)	2.67	0.177
(42324 3) - (42268 1)	0.00205	0.00461	(-0.01267, 0.01677)	0.45	1.000
(42268 3) - (42268 2)	0.00670	0.00461	(-0.00802, 0.02142)	1.46	0.872
(42296 1) - (42268 2)	**0.01494**	**0.00461**	**(0.00022, 0.02966)**	**3.24**	**0.044**
(42296 2) - (42268 2)	0.00716	0.00461	(-0.00756, 0.02188)	1.55	0.825
(42296 3) - (42268 2)	0.00967	0.00461	(-0.00505, 0.02439)	2.10	0.482
(42324 1) - (42268 2)	0.01404	0.00461	(-0.00068, 0.02876)	3.05	0.074
(42324 2) - (42268 2)	0.01337	0.00461	(-0.00135, 0.02809)	2.90	0.105
(42324 3) - (42268 2)	0.00312	0.00461	(-0.01160, 0.01784)	0.68	0.999
(42296 1) - (42268 3)	0.00824	0.00461	(-0.00648, 0.02296)	1.79	0.689
(42296 2) - (42268 3)	0.00046	0.00461	(-0.01426, 0.01518)	0.10	1.000
(42296 3) - (42268 3)	0.00297	0.00461	(-0.01175, 0.01769)	0.64	0.999
(42324 1) - (42268 3)	0.00734	0.00461	(-0.00738, 0.02206)	1.59	0.805
(42324 2) - (42268 3)	0.00667	0.00461	(-0.00805, 0.02139)	1.45	0.875
(42324 3) - (42268 3)	-0.00358	0.00461	(-0.01830, 0.01114)	-0.78	0.997
(42296 2) - (42296 1)	-0.00778	0.00461	(-0.02250, 0.00694)	-1.69	0.751
(42296 3) - (42296 1)	-0.00527	0.00461	(-0.01999, 0.00945)	-1.15	0.965
(42324 1) - (42296 1)	-0.00090	0.00461	(-0.01562, 0.01381)	-0.20	1.000
(42324 2) - (42296 1)	-0.00157	0.00461	(-0.01629, 0.01315)	-0.34	1.000
(42324 3) - (42296 1)	-0.01183	0.00461	(-0.02655, 0.00289)	-2.57	0.219
(42296 3) - (42296 2)	0.00251	0.00461	(-0.01221, 0.01723)	0.54	1.000
(42324 1) - (42296 2)	0.00688	0.00461	(-0.00784, 0.02160)	1.49	0.855
(42324 2) - (42296 2)	0.00621	0.00461	(-0.00851, 0.02093)	1.35	0.913
(42324 3) - (42296 2)	-0.00404	0.00461	(-0.01876, 0.01068)	-0.88	0.993
(42324 1) - (42296 3)	0.00437	0.00461	(-0.01035, 0.01909)	0.95	0.989
(42324 2) - (42296 3)	0.00370	0.00461	(-0.01102, 0.01842)	0.80	0.996
(42324 3) - (42296 3)	-0.00655	0.00461	(-0.02127, 0.00817)	-1.42	0.885
(42324 2) - (42324 1)	-0.00067	0.00461	(-0.01539, 0.01405)	-0.14	1.000
(42324 3) - (42324 1)	-0.01092	0.00461	(-0.02564, 0.00380)	-2.37	0.315
(42324 3) - (42324 2)	-0.01025	0.00461	(-0.02497, 0.00447)	-2.23	0.400

Table II.11

Tukey Pairwise Comparisons for Mean Cup Shape
Single Factor of Ploidy

Grouping Information Using the Tukey Method and 95% Confidence

PLOIDY	N	Mean	Grouping
D	54	0.363725	A
T	54	0.345274	B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of PLOIDY Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
T - D	-0.01845	0.00217	(-0.02278, -0.01412)	-8.50	0.000

Table II.12

Tukey Pairwise Comparisons for Mean Fan Shape
Interaction of Sample Date*Ploidy

Grouping Information Using the Tukey Method and 95% Confidence

SAMPLE DATE*PLOIDY	N	Mean	Grouping		
42324 D	18	0.723397	A		
42296 D	18	0.707379	A	B	
42268 D	18	0.707190	A	B	
42268 T	18	0.700684		B	C
42296 T	18	0.696618		B	C
42324 T	18	0.685354			C

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of SAMPLE DATE*PLOIDY Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted pValue
(42268 T) - (42268 D)	-0.00651	0.00636	(-0.02513, 0.01212)	-1.02	0.909
(42296 D) - (42268 D)	0.00019	0.00636	(-0.01844, 0.01882)	0.03	1.000
(42296 T) - (42268 D)	-0.01057	0.00636	(-0.02920, 0.00806)	-1.66	0.561
(42324 D) - (42268 D)	0.01621	0.00636	(-0.00242, 0.03483)	2.55	0.124
(42324 T) - (42268 D)	**-0.02184**	**0.00636**	**(-0.04046, -0.00321)**	**-3.43**	**0.012**
(42296 D) - (42268 T)	0.00669	0.00636	(-0.01193, 0.02532)	1.05	0.898
(42296 T) - (42268 T)	-0.00407	0.00636	(-0.02269, 0.01456)	-0.64	0.988
(42324 D) - (42268 T)	**0.02271**	**0.00636**	**(0.00409, 0.04134)**	**3.57**	**0.008**
(42324 T) - (42268 T)	-0.01533	0.00636	(-0.03396, 0.00330)	-2.41	0.167
(42296 T) - (42296 D)	-0.01076	0.00636	(-0.02939, 0.00787)	-1.69	0.542
(42324 D) - (42296 D)	0.01602	0.00636	(-0.00261, 0.03465)	2.52	0.133
(42324 T) - (42296 D)	**-0.02202**	**0.00636**	**(-0.04065, -0.00340)**	**-3.46**	**0.011**
(42324 D) - (42296 T)	**0.02678**	**0.00636**	**(0.00815, 0.04541)**	**4.21**	**0.001**
(42324 T) - (42296 T)	-0.01126	0.00636	(-0.02989, 0.00736)	-1.77	0.491
(42324 T) - (42324 D)	**-0.03804**	**0.00636**	**(-0.05667, -0.01942)**	**-5.98**	**0.000**

Table II.13

Tukey Pairwise Comparisons for Mean Delta Condition Index
Interaction of Sample Date*Ploidy*Flip

Grouping Information Using the Tukey Method and 95% Confidence

SAMPLE DATE*PLOIDY*FLIP	N	Mean	Grouping
42268 T W	9	15.4353	A
42324 T B	9	15.3596	A
42268 T B	9	14.6985	A B
42296 T W	9	14.0677	A B C
42324 D W	9	12.7413	A B C D
42296 T B	9	12.5882	B C D
42324 T W	9	11.8528	C D E
42268 D W	9	10.4014	D E
42296 D B	9	10.0974	D E F
42324 D B	9	9.8595	E F
42296 D W	9	9.3095	E F
42268 D B	9	7.5024	F

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of SAMPLE DATE*PLOIDY*FLIP Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(42268 D W) - (42268 D B)	2.899	0.803	(0.184, 5.614)	3.61	0.026
(42268 T B) - (42268 D B)	7.196	0.803	(4.481, 9.911)	8.96	0.000
(42268 T W) - (42268 D B)	7.933	0.803	(5.218, 10.648)	9.88	0.000
(42296 D B) - (42268 D B)	**2.595**	**0.803**	**(-0.120, 5.310)**	**3.23**	**0.074**
(42296 D W) - (42268 D B)	**1.807**	**0.803**	**(-0.908, 4.522)**	**2.25**	**0.521**
(42296 T B) - (42268 D B)	5.086	0.803	(2.371, 7.801)	6.33	0.000
(42296 T W) - (42268 D B)	6.565	0.803	(3.850, 9.280)	8.17	0.000
(42324 D B) - (42268 D B)	**2.357**	**0.803**	**(-0.358, 5.072)**	**2.93**	**0.151**
(42324 D W) - (42268 D B)	5.239	0.803	(2.524, 7.954)	6.52	0.000
(42324 T B) - (42268 D B)	7.857	0.803	(5.142, 10.572)	9.78	0.000
(42324 T W) - (42268 D B)	4.350	0.803	(1.635, 7.065)	5.42	0.000
(42268 T B) - (42268 D W)	4.297	0.803	(1.582, 7.012)	5.35	0.000
(42268 T W) - (42268 D W)	5.034	0.803	(2.319, 7.749)	6.27	0.000
(42296 D B) - (42268 D W)	**-0.304**	**0.803**	**(-3.019, 2.411)**	**-0.38**	**1.000**
(42296 D W) - (42268 D W)	**-1.092**	**0.803**	**(-3.807, 1.623)**	**-1.36**	**0.967**
(42296 T B) - (42268 D W)	**2.187**	**0.803**	**(-0.528, 4.902)**	**2.72**	**0.237**
(42296 T W) - (42268 D W)	3.666	0.803	(0.951, 6.381)	4.56	0.001
(42324 D B) - (42268 D W)	**-0.542**	**0.803**	**(-3.257, 2.173)**	**-0.67**	**1.000**
(42324 D W) - (42268 D W)	**2.340**	**0.803**	**(-0.375, 5.055)**	**2.91**	**0.159**
(42324 T B) - (42268 D W)	4.958	0.803	(2.243, 7.673)	6.17	0.000
(42324 T W) - (42268 D W)	**1.451**	**0.803**	**(-1.264, 4.166)**	**1.81**	**0.809**
(42268 T W) - (42268 T B)	**0.737**	**0.803**	**(-1.978, 3.452)**	**0.92**	**0.999**
(42296 D B) - (42268 T B)	-4.601	0.803	(-7.316, -1.886)	-5.73	0.000
(42296 D W) - (42268 T B)	-5.389	0.803	(-8.104, -2.674)	-6.71	0.000
(42296 T B) - (42268 T B)	**-2.110**	**0.803**	**(-4.825, 0.605)**	**-2.63**	**0.285**
(42296 T W) - (42268 T B)	**-0.631**	**0.803**	**(-3.346, 2.084)**	**-0.79**	**1.000**
(42324 D B) - (42268 T B)	-4.839	0.803	(-7.554, -2.124)	-6.02	0.000
(42324 D W) - (42268 T B)	**-1.957**	**0.803**	**(-4.672, 0.758)**	**-2.44**	**0.396**
(42324 T B) - (42268 T B)	**0.661**	**0.803**	**(-2.054, 3.376)**	**0.82**	**1.000**
(42324 T W) - (42268 T B)	-2.846	0.803	(-5.561, -0.131)	-3.54	0.032
(42296 D B) - (42268 T W)	-5.338	0.803	(-8.053, -2.623)	-6.65	0.000
(42296 D W) - (42268 T W)	-6.126	0.803	(-8.841, -3.411)	-7.63	0.000
(42296 T B) - (42268 T W)	-2.847	0.803	(-5.562, -0.132)	-3.54	0.031
(42296 T W) - (42268 T W)	**-1.368**	**0.803**	**(-4.083, 1.347)**	**-1.70**	**0.861**
(42324 D B) - (42268 T W)	-5.576	0.803	(-8.291, -2.861)	-6.94	0.000
(42324 D W) - (42268 T W)	-2.694	0.803	(-5.409, 0.021)	-3.35	0.053
(42324 T B) - (42268 T W)	**-0.076**	**0.803**	**(-2.791, 2.639)**	**-0.09**	**1.000**
(42324 T W) - (42268 T W)	-3.583	0.803	(-6.298, -0.868)	-4.46	0.002
(42296 D W) - (42296 D B)	**-0.788**	**0.803**	**(-3.503, 1.927)**	**-0.98**	**0.998**
(42296 T B) - (42296 D B)	**2.491**	**0.803**	**(-0.224, 5.206)**	**3.10**	**0.102**
(42296 T W) - (42296 D B)	3.970	0.803	(1.255, 6.685)	4.94	0.000
(42324 D B) - (42296 D B)	**-0.238**	**0.803**	**(-2.953, 2.477)**	**-0.30**	**1.000**
(42324 D W) - (42296 D B)	**2.644**	**0.803**	**(-0.071, 5.359)**	**3.29**	**0.063**
(42324 T B) - (42296 D B)	5.262	0.803	(2.547, 7.977)	6.55	0.000
(42324 T W) - (42296 D B)	**1.755**	**0.803**	**(-0.960, 4.470)**	**2.19**	**0.565**
(42296 T B) - (42296 D W)	3.279	0.803	(0.564, 5.994)	4.08	0.006
(42296 T W) - (42296 D W)	4.758	0.803	(2.043, 7.473)	5.92	0.000
(42324 D B) - (42296 D W)	**0.550**	**0.803**	**(-2.165, 3.265)**	**0.68**	**1.000**
(42324 D W) - (42296 D W)	3.432	0.803	(0.717, 6.147)	4.27	0.003
(42324 T B) - (42296 D W)	6.050	0.803	(3.335, 8.765)	7.53	0.000
(42324 T W) - (42296 D W)	**2.543**	**0.803**	**(-0.172, 5.258)**	**3.17**	**0.087**
(42296 T W) - (42296 T B)	**1.479**	**0.803**	**(-1.235, 4.194)**	**1.84**	**0.789**
(42324 D B) - (42296 T B)	-2.729	0.803	(-5.444, -0.014)	-3.40	0.047
(42324 D W) - (42296 T B)	**0.153**	**0.803**	**(-2.562, 2.868)**	**0.19**	**1.000**
(42324 T B) - (42296 T B)	2.771	0.803	(0.056, 5.486)	3.45	0.041
(42324 T W) - (42296 T B)	**-0.735**	**0.803**	**(-3.450, 1.979)**	**-0.92**	**0.999**
(42324 D B) - (42296 T W)	-4.208	0.803	(-6.923, -1.493)	-5.24	0.000
(42324 D W) - (42296 T W)	**-1.326**	**0.803**	**(-4.041, 1.389)**	**-1.65**	**0.883**
(42324 T B) - (42296 T W)	**1.292**	**0.803**	**(-1.423, 4.007)**	**1.61**	**0.900**
(42324 T W) - (42296 T W)	**-2.215**	**0.803**	**(-4.930, 0.500)**	**-2.76**	**0.221**
(42324 D W) - (42324 D B)	2.882	0.803	(0.167, 5.597)	3.59	0.028
(42324 T B) - (42324 D B)	5.500	0.803	(2.785, 8.215)	6.85	0.000
(42324 T W) - (42324 D B)	**1.993**	**0.803**	**(-0.722, 4.708)**	**2.48**	**0.368**
(42324 T B) - (42324 D W)	**2.618**	**0.803**	**(-0.097, 5.333)**	**3.26**	**0.069**
(42324 T W) - (42324 D W)	**-0.889**	**0.803**	**(-3.603, 1.826)**	**-1.11**	**0.993**
(42324 T W) - (42324 T B)	-3.507	0.803	(-6.222, -0.792)	-4.37	0.002

Table II.14

Tukey Pairwise Comparisons for Mean Delta Condition Index
Interaction of Ploidy*Stocking Density

Grouping Information Using the Tukey Method and 95% Confidence

PLOIDY*STOCKING DENSITY	N	Mean	Grouping		
T 1	18	15.0358	A		
T 2	18	13.6806	A	B	
T 3	18	13.2846		B	
D 2	18	10.3662			C
D 3	18	10.0213			C
D 1	18	9.5681			C

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of PLOIDY*STOCKING DENSITY Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(D 2) - (D 1)	**0.798**	**0.568**	**(-0.865, 2.461)**	**1.41**	**0.724**
(D 3) - (D 1)	**0.453**	**0.568**	**(-1.209, 2.116)**	**0.80**	**0.967**
(T 1) - (D 1)	5.468	0.568	(3.805, 7.130)	9.63	0.000
(T 2) - (D 1)	4.112	0.568	(2.450, 5.775)	7.24	0.000
(T 3) - (D 1)	3.716	0.568	(2.054, 5.379)	6.54	0.000
(D 3) - (D 2)	**-0.345**	**0.568**	**(-2.008, 1.318)**	**-0.61**	**0.990**
(T 1) - (D 2)	4.670	0.568	(3.007, 6.332)	8.22	0.000
(T 2) - (D 2)	3.314	0.568	(1.652, 4.977)	5.84	0.000
(T 3) - (D 2)	2.918	0.568	(1.256, 4.581)	5.14	0.000
(T 1) - (D 3)	5.014	0.568	(3.352, 6.677)	8.83	0.000
(T 2) - (D 3)	3.659	0.568	(1.997, 5.322)	6.44	0.000
(T 3) - (D 3)	3.263	0.568	(1.601, 4.926)	5.75	0.000
(T 2) - (T 1)	**-1.355**	**0.568**	**(-3.018, 0.308)**	**-2.39**	**0.175**
(T 3) - (T 1)	-1.751	0.568	(-3.414, -0.089)	-3.08	0.033
(T 3) - (T 2)	**-0.396**	**0.568**	**(-2.059, 1.267)**	**-0.70**	**0.982**

Table II.15

Tukey Pairwise Comparisons for Mean Ash Free Dry Weight (MAFDW)
Interaction of Flip*Stocking Density

Grouping Information Using the Tukey Method and 95% Confidence

Flip*Stock Density	N	Mean	Grouping		
B 1	18	0.0864556	A		
B 3	18	0.0423778		B	
B 2	18	0.0238444		B	C
W 3	18	0.0025000			C
W 2	18	0.0024167			C
W 1	18	0.0017278			C

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Flip*Stock Density Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(B 2) - (B 1)	-0.0626	0.0127	(-0.0994, -0.0258)	-4.95	0.000
(B 3) - (B 1)	-0.0441	0.0127	(-0.0808, -0.0073)	-3.48	0.009
(W 1) - (B 1)	-0.0847	0.0127	(-0.1215, -0.0480)	-6.70	0.000
(W 2) - (B 1)	-0.0840	0.0127	(-0.1208, -0.0473)	-6.64	0.000
(W 3) - (B 1)	-0.0840	0.0127	(-0.1207, -0.0472)	-6.64	0.000
(B 3) - (B 2)	**0.0185**	**0.0127**	**(-0.0182, 0.0553)**	**1.46**	**0.687**
(W 1) - (B 2)	**-0.0221**	**0.0127**	**(-0.0589, 0.0147)**	**-1.75**	**0.504**
(W 2) - (B 2)	**-0.0214**	**0.0127**	**(-0.0582, 0.0153)**	**-1.69**	**0.539**
(W 3) - (B 2)	**-0.0213**	**0.0127**	**(-0.0581, 0.0154)**	**-1.69**	**0.544**
(W 1) - (B 3)	-0.0406	0.0127	(-0.0774, -0.0039)	-3.21	0.021
(W 2) - (B 3)	-0.0400	0.0127	(-0.0767, -0.0032)	-3.16	0.025
(W 3) - (B 3)	-0.0399	0.0127	(-0.0766, -0.0031)	-3.15	0.026
(W 2) - (W 1)	**0.0007**	**0.0127**	**(-0.0361, 0.0375)**	**0.05**	**1.000**
(W 3) - (W 1)	**0.0008**	**0.0127**	**(-0.0360, 0.0375)**	**0.06**	**1.000**
(W 3) - (W 2)	**0.0001**	**0.0127**	**(-0.0367, 0.0369)**	**0.01**	**1.000**

Table II.16

Tukey Pairwise Comparisons for Mean Percent Mortality
Single Factor of Ploidy

Grouping Information Using the Tukey Method and 95% Confidence

Ploidy	N	Mean	Grouping	
D	18	0.0687090	A	
T	18	0.0279471		B

Means that do not share a letter are significantly different.

Appendix III
Chapter 4 Supporting Data

Table III. 1

Tukey Pairwise Comparisons for Mean Worm Count
Interaction of Ploidy*Flip

Grouping Information Using the Tukey Method and 95% Confidence

PLOIDY*FLIP	N	Mean	Grouping
D B	27	130.593	A
T B	27	66.259	B
D W	27	3.222	C
T W	27	1.926	C

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of PLOIDY*FLIP Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(D W) - (D B)	-127.4	17.9	(-174.2, -80.5)	-7.12	0.000
(T B) - (D B)	-64.3	17.9	(-111.2, -17.5)	-3.59	0.003
(T W) - (D B)	-128.7	17.9	(-175.5, -81.8)	-7.19	0.000
(T B) - (D W)	63.0	17.9	(16.2, 109.9)	3.52	0.004
(T W) - (D W)	**-1.3**	**17.9**	**(-48.1, 45.5)**	**-0.07**	**1.000**
(T W) - (T B)	-64.3	17.9	(-111.2, -17.5)	-3.59	0.003

Table III. 2

Tukey Pairwise Comparisons for Mean Dry Worm Weight
Interaction of Ploidy*Flip

Grouping Information Using the Tukey Method and 95% Confidence

PLOIDY*FLIP	N	Mean	Grouping	
D B	27	0.0862111	A	
T B	27	0.0366926		B
D W	27	0.0016148	B	C
T W	27	0.0006222		C

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of PLOIDY*FLIP Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(D W) - (D B)	-0.0846	0.0136	(-0.1205, -0.0487)	-6.20	0.000
(T B) - (D B)	-0.0495	0.0136	(-0.0854, -0.0136)	-3.63	0.003
(T W) - (D B)	-0.0856	0.0136	(-0.1215, -0.0497)	-6.28	0.000
(T B) - (D W)	0.0351	0.0136	(-0.0008, 0.0710)	2.57	0.058
(T W) - (D W)	-0.0010	0.0136	(-0.0369, 0.0349)	-0.07	1.000
(T W) - (T B)	-0.0361	0.0136	(-0.0719, -0.0002)	-2.64	0.048

Table III. 3

Tukey Pairwise Comparisons for Mean Dry Worm Weight
Interaction of SampleDate*Flip

Grouping Information Using the Tukey Method and 95% Confidence

SAMPLE_DATE*FLIP	N	Mean	Grouping
42324 B	18	0.123344	A
42296 B	18	0.041056	B
42268 B	18	0.019956	B
42324 W	18	0.001394	B
42268 W	18	0.001133	B
42296 W	18	0.000828	B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of SAMPLE_DATE*FLIP Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(42268 W) - (42268 B)	-0.0188	0.0167	(-0.0677, 0.0301)	-1.13	0.869
(42296 B) - (42268 B)	0.0211	0.0167	(-0.0278, 0.0700)	1.26	0.804
(42296 W) - (42268 B)	-0.0191	0.0167	(-0.0680, 0.0298)	-1.15	0.861
(42324 B) - (42268 B)	0.1034	0.0167	(0.0545, 0.1523)	6.19	0.000
(42324 W) - (42268 B)	-0.0186	0.0167	(-0.0675, 0.0303)	-1.11	0.875
(42296 B) - (42268 W)	0.0399	0.0167	(-0.0090, 0.0888)	2.39	0.174
(42296 W) - (42268 W)	-0.0003	0.0167	(-0.0492, 0.0486)	-0.02	1.000
(42324 B) - (42268 W)	0.1222	0.0167	(0.0733, 0.1711)	7.32	0.000
(42324 W) - (42268 W)	0.0003	0.0167	(-0.0486, 0.0492)	0.02	1.000
(42296 W) - (42296 B)	-0.0402	0.0167	(-0.0891, 0.0087)	-2.41	0.167
(42324 B) - (42296 B)	0.0823	0.0167	(0.0334, 0.1312)	4.93	0.000
(42324 W) - (42296 B)	-0.0397	0.0167	(-0.0886, 0.0092)	-2.37	0.179
(42324 B) - (42296 W)	0.1225	0.0167	(0.0736, 0.1714)	7.33	0.000
(42324 W) - (42296 W)	0.0006	0.0167	(-0.0483, 0.0495)	0.03	1.000
(42324 W) - (42324 B)	-0.1220	0.0167	(-0.1709, -0.0730)	-7.30	0.000